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# Guidance for the Reregistration of Pesticide Products Containing Cycloheximide

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# GUIDANCE FOR THE REREGISTRATION OF PESTICIDE PRODUCTS

# CONTAINING

# CYCLOHEXIMIDE

3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxethyl] glutarimide

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS

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# CHAPTER I: HOW TO REGISTER UNDER A REGISTRATION STANDARD

## A. Organization of the Standard

This first chapter explains the purpose of a Registration Standard and summarizes the legal principles involved in registering or reregistering under a Standard. The second chapter involves the regulatory position and the rationale supporting this position. The third chapter sets forth the data requirements that must be met to obtain or retain registration for products covered by this particular Registration Standard.

## B. Purpose of the Standard

Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provides that "no person in any State may distribute, sell, offer for sale, hold for sale, ship, deliver for shipment, or receive (and having so received) deliver or offer to deliver, to any person any pesticide which is not registered with the Administrator [of EPA]." To approve the registration of a pesticide, the Administrator must find, pursuant to Section 3(c)(5) that:

> "(A) its composition is such as to warrant the proposed claims for it;
>
> (B) its labeling and othe material required to be submitted comply with the requirements of this Act;
>
> (C) it will perform its intended function without unreasonable adverse effects on the environment; and
>
> (D) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

In making these findings, the Agency reviews a wide range of data which registrants are required to submit and assesses the risks and benefits associated with the use of the proposed pesticide. However, the established approach to making these findings has been found to be defective on two counts.

First, EPA and its predecessor agency, the United States Department of Agriculture (USDA), routinely reviewed registration applications on "product by product" basis, evaluating each product-specific application somewhat independently. In the review of products containing similar components, there was little opportunity for a retrospective review of the full range of pertinent data available in Agency files and in the public literature. Thus the "product by product" approach was often inefficient and sometimes resulted in inconsistent or incomplete regulatory judgments.

Second, over the years, as a result of inevitable and continuing advances in scientific knowledge, methodology, and policy, the data base for many pesticides came to be considered inadequate by current scientific and regulatory standards. Given the long history of pesticide regulation in several agencies, it is even likely that materials may have been lost from the data files. When EPA issued new requirements for registration in 1975 (40 CFR 162) and proposed new guidelines for hazard testing in 1978 (43 FR 2986, July 10, 1978 and 43 FR 37336, August 22, 1978), many products that had already been registered for years were being sold and used without the same assurances of human and environmental safety as was being required for new products. Because of this inconsistency, Congress directed EPA to reregister all previously registered products, so as to bring their registrations and their data bases into compliance with current requirements [See FIFRA Section 3(g)].

Facing the enormous job of re-reviewing and calling-in new data for the approximately 35,000 current registrations, and realizing the inefficiencies of the "product by product" approach, the Agency decided that a new, more effective method of review was needed.

A new review procedure has been developed. Under it, EPA publishes documents called Registration Standards, each of which discusses a particular pesticide active ingredient. Each Registration Standard summarizes all the data available to the Agency on a particular active ingredient and its current uses, and sets forth the Agency's comprehensive position on the conditions and requirements for registation of all existing and future products which contain that active ingredient. These conditions and requirements, all of which must be met to obtain or retain full registration or reregistration under Section 3(c) (5) of FIFRA, include the submission of needed scientific data which the Agency does not now have, compliance with standards of toxicity, composition, labeling, and packaging, and satisfaction of the data compensation provisions of FIFRA Section 3(c)(1)(D).

The Standard will also serve as a tool for product classification. As part of the registration of a pesticide product, EPA may classify each product for "general use" or "restricted use" [FIFRA Section 3(d)]. A pesticide is classified for "restricted use" when some special regulatory restriction is needed to ensure against unreasonable adverse effects to man or the environment. Many such risks of unreasonable edverse effects can be lessened if expressly-designed label precautions are strictly followed. Thus the special regulatory restriction for a "restricted use" pesticide is usually a requirement that it be applied only by, or under the supervision of, an applicator who has been certified by the State or Federal government as being competent to use the pesticide safely, responsibly, and in accordance with label directions. A restricted-use pesticide can have other regulatory restrictions [40 CFR 162.11(c)(5)] instead of, or in addition to, the certified applicator requirement. These other regulatory restrictions may include such actions as seasonal or regional limitations on use, or a requirement for the monitoring of the pesticide with risk criteria listed in 40 CFR 162.11(c), and thereby residue levels after use. A pesticide classified for "general use," or not classified at all, is available for use by any individual who is in compliance with State or local regulations. The Registration Standard review compares information about potential adverse effects of specific uses of the pesticide with risk criteria listed in 40 CFR 162.11(c), and thereby determines whether a product needs to be classified for "restricted use." If the Standard does classify a pesticide for "restricted use," this determination is stated in the second chapter.

The Standard will directly register or reregister only manufacturing-use products and their intermediaries. End-use products will be registered or reregistered under a separate Label Improvement Program. The only exception to this general rule will arise when there is no manufacturing-use product registered for a particular end-use product. In this case, the end-use product will be treated as if it were a manufacturing-use product.

The Agency has separated the reregistration of end-use products from that of manufacturing-use products in order to increase the rapidity with which products may be reregistered and to place emphasis on the concern for chronic health effects data which prompted the legislative requirement for reregistration. The utility of this approach is the product of two factors. First, end-use product specific data requirements are minimal and can be more effectively handled through a separate process. Second, a separate process obviates many of the difficulties inherent in reregistration of products which are mixtures of more than one active ingredient and will ultimately be affected by several Standards. Under the Label Improvement Program, end-use products will be reregistered on a separate schedule from the reregistration of manufacturing-use products.

The Standard will consider all uses of the active ingredient which are registered for end-use products at the time the Standard is developed, including use patterns which pertain only to mixtures. The Standard will also establish labeling requirements for manufacturing-use products which formulators of end-use products will have to follow. However, the imposition of these labeling requirements directly on formulators will fall under the Label Improvement Program.

## C. Requirement to Reregister under the Standard

FIFRA Section 3(g), as amended in 1978, directs EPA to reregister all currently registered products as expeditiously as possible. Congress also agreed that reregistration should be accomplished by the use of Registration Standards.

Each registrant of a currently registered product to which this Standard applies, and who wishes to continue to sell or distribute his product in commerce, must apply for reregistration. His application must contain proposed labeling that complies with this Standard.

EPA will issue a notice of intent to cancel the registration of any currently registered product to which this Standard applies if the registrant fails to comply with procedures for reregistration set forth in the Guidance Package which accompanies this Standard.

## D. "Product Specific" Data and "Generic" Data

In the course of developing this Standard, EPA has determined the types of data needed for evaluation of the properties and effects of products to which the Standard applies in the disciplinary areas of Product Chemistry Environmental Fate, Toxicology, Residue Chemistry, and Ecological Effects. These determinations are based primarily on the data Guidelines proposed in 43 FR 29696, July 10, 1978; 43 FR 37336, August 22, 1978; and 45 FR 72948, November 3, 1980, as applied to the use patterns of the products to which this Standard applies. Where it appeared that data from a normally applicable Guidelines requirement was actually unnecessary to evaluate these products, the Standard indicates that the requirement has been waived. On the other hand, in some cases studies not required by the Guidelines may be needed because of the particular composition or use pattern of products the Standard covers; if so, the Standard explains the Agency's reasoning. Data guidelines have not yet been proposed for the Residue Chemistry discipline, but the requirements for such data have been in effect for some time and are, the Agency believes, relatively familiar to registrants. Data which we have found are needed to evaluate the registrability of some products covered by the Standard may not be needed for the evaluation of other products, depending upon the composition, formulation type, and intended uses of the product in question. The Standard states which data requirements apply to which product categories. (See the third chapter.) The various kinds of data normally required for registration of a pesticide product can be divided into two basic groups:

1. Data that are product-specific, i.e., data that relate only to the properties or effects of a product with a particular composition (or a group of products with closely similar composition); and

2. Generic data that pertains to the properties or effects of a particular ingredient, and thus are relevant to an evaluation of the risks and benefits of all products containing that ingredient (or all such products having a certain use pattern), regardless of any such product's unique composition.

The Agency requires certain "product-specific" data for each product to characterize the product's particular composition and physical/chemical properties (Product Chemistry), and to characterize the product's acute toxicity (which is a function of its total composition). The applicant for registration or reregistration of any manufacturing-use product, and without regard to its intended use pattern, must submit or cite enough of these kinds of data to allow EPA to evaluate the product. For such purposes, "product-specific" data on any product other than the applicant's are irrelevant, unless the other product is closely similar in composition to the applicant's. (Where it has been found practicable to group similar products for purposes of evaluating, with a single set of tests, all products in the group, the Standard so indicates.)

All other data needed to evaluate pesticide products concern the properties or effects of a particular ingredient of products (normally a pesticidally active ingredient, but in some cases a pesticidally inactive, or "inert," ingredient). Some data in this "generic" category are required to evaluate the properties and effects of all products containing that ingredient [e.g., the acute $LD_{50}$ of the active ingredient in its technical or purer grade; see proposed guidelines, 43 FR 37355].

Other "generic" data are required to evaluate all products which both contain a particular ingredient and are intended for certain uses (see, e.g., proposed 40 CFR 163.82-1, 43 FR 37363, which requires subchronic oral testing of the active ingredient with respect to certain use patterns only). Where a particular data requirement is use-pattern dependent, it will apply to each manufacturing-use product with labeling that allows it to be used to make end-use products with that use pattern. Thus, for example, a subchronic oral dosing study is needed to evaluate the safety of any manufacturing-use product that legally could be used to make an end-use, food-crop pesticide.

If a registrant of a currently registered manufacturing-use product wishes to avoid the costs of data compensation [under FIFRA Section 3(c)(1)(D)] or data generation [under Section 3(c)(2)(B)] for "generic" data that are required only with respect to some use patterns, he may elect to delete those use patterns from his labeling at the time he reregisters his product. An applicant for registration of a new product under this Standard may similarly request approval for only certain use patterns.

## E. Data Compensation Requirements under FIFRA 3(c)(1)(D)

Under FIFRA Section 3(c)(1)(D), an applicant for registration, reregistration, or amended registration must offer to pay compensation for certain existing data the Agency has used in developing the Registration Standard. The data for which compensation must be offered are all data which are described by all of the following criteria:

1. The data were first submitted to EPA (or to its predecessor agencies, USDA OR FDA), on or after January 1, 1979;
2. The data were fist submitted to EPA (or USDA or FDA) by some other applicant or registrant in support of an application for an experimental use permit, an amendment adding a new use to a registration, or for registration, or to support or maintain an existing registration;
3. They are the kind of data which are relevant to the Agency's decision to register or reregister the applicant's product under the Registration Standard, taking into account the applicant's product's composition and intended use pattern(s);
4. The Agency has found the data to be valid and usable in reaching regulatory conclusions.

An applicant for reregistration of an already registered product under this Standard, or for registration of a new product under this Standard, accordingly must determine which of the data used by EPA in developing the Standard must be the subject of an offer to pay compensation, and must submit with his application the appropriate statements evidencing his compliance with FIFRA Section 3(c)(1)(D).

An applicant would never be required to offer to pay for "product-specific" data submitted by another firm. In many, if not in most cases, data which are specific to another firm's product will not suffice to allow EPA to evaluate the applicant's product, that is, will not be useful to the Agency in determining whether the applicant's product is registrable. There may be cases, however, where because of close similarities between the composition of two or more products, another firm's data may suffice to allow EPA to evaluate some or all of the "product-specific" aspects of the applicant's product. In such a case, the applicant may choose to cite that data instead of submitting data from tests on his own product, and if he chooses that option, he would have to comply with the offer-to-pay requirements of Section 3(c)(1)(D) for those data.

Each applicant for registration or reregistration of a manufacturing-use product must comply with the Section 3(c)(1)(D) requirements with respect to each item of "generic" data that relates to his product's intended uses.

A detailed description of the procedures an applicant must follow in applying for reregistration (or new registration) under this Standard is found in the Guidance Package for this Standard.

### F. Obtaining Data to Fill "Data Gaps"; FIFRA 3(c)(2)(B)

Some of the kinds of data EPA needs for its evaluation of the properties and effects of products to which this Standard applies have never been submitted to the Agency (or, if submitted, have been found to have deficiencies rendering them inadequate for making registrability decisions) and have not been located in the published literature search that EPA conducted as part of preparing this Standard. Such instances of missing but required data are referred to in the Standard as "data gaps."

FIFRA Section 3(c)(2)(B), added to FIFRA by the Congress in 1978, authorizes EPA to require registrants to whom a data requirement applies to generate (or otherwise produce) data to fill such "gaps" and submit those data to EPA. EPA must allow a reasonably sufficient period for this to be accomplished. If a registrant fails to take appropriate and timely steps to fill the data gaps identified by a section 3(c)(2)(B) order, his product's registration may be suspended until the data are submitted. A mechanism is provided whereby two or more registrants may agree to share in the costs of producing data for which they are both responsible.

The Standard lists, in the third chapter, the "generic" data gaps and notes the classes of products to which these data gaps pertain. The Standard also points out that to be registrable under the Standard, a product must be supported by certain required "product-specific" data.

As part of the process of reregistering currently registered products, EPA will issue Section 3(c)(2)(B) directives requiring the registrants to take appropriate steps to fill all identified data gaps -- whether the data in question are "product-specific" or "generic" -- in accordance with a schedule. Registrants are reminded that Section 6(a)(2) of FIFRA requires you at any time to submit factual information raising concerns of possible unreasonable adverse effects of a pesticide. You should notify the Agency of interim results of studies in progress if those results show possible adverse effects.

## G. Amendments to the Standard

Applications for registration which propose uses or formulations that are not presently covered by the Standard, or which present product compositions, product chemistry data, hazard data, toxicity levels, or labeling that do not meet the requirements of the Standard, will automatically be considered by the Agency to be requests for amendments to the Standard. In response to such applications, the Agency may request additional data to support the proposed amendment to the Standard, or may deny the application for registration on the grounds that the proposed product would cause unreasonable adverse effects to the environment. In the former case, when additional data have been satisfactorily supplied, and providing that the data do not indicate the potential for unreasonable adverse effects, the Agency will then amend the Standard to cover the new registration.

Each Registration Standard is based upon all data and information available to the Agency's reviewers on a particular date prior to the publication date. This "cut-off" date is stated at the beginning of the second chapter. Any subsequent data submissions and any approved amendments will be incorporated into the Registration Standard by means of addenda, which are available for inspection at EPA in Washington, D.C., or copies of which may be requested from the Agency.

While the Registration Standard discusses only the uses and hazards of products containing the designated active ingredient(s), the Agency is also concerned with the potential hazards of some inert ingredients and impurities. Independent of the development of any one Atandard, the Agency has initiated the evaluation of some inert pesticide ingredients. Where the Agency has identified inert ingredients of concern in a specific product to which the Standard applies, these ingredients will be pointed out in the Guidance Package.

## CHAPTER II. REGULATORY POSITION AND RATIONALE

### A. Introduction

This chapter describes the Agency's regulatory position on manufacturing-use products which either contain cycloheximide as the sole active ingredient or in mixtures with other active ingredients. The Agency's regulatory position on cycloheximide incorporates a number of considerations. Foremost among these considerations is an analysis of the registrability of products containing cycloheximide based on the risk criteria found in Section 162.11(a) of Title 40 of the U.S. Code of Federal Regulations.

In addition to the basic regulatory decision and rationale, this chapter includes the following: the criteria which will be used in evaluating applications for registration of manufacturing-use pesticide products that contain cycloheximide as one of the active ingredients, appropriate labeling for those products, and requirements related to tolerance reassessment, if applicable.

The data requirements which must be met to continue registration of cycloheximide products are presented in tabular form in Chapter III: Summary of Data Requirements and Data Gaps. The rationale for requiring specific data is given in the footnotes to the tables. The footnotes will also explain any requirements that differ from those presented in the Agency guidelines for the registration of pesticides.

### B. Description of the Chemical

Cycloheximide is a fungicide and a growth regulator (abscission agent) which is biologically synthesized. As a fungicide, it is applied at relatively low dosages (40 g/acre or less) to control fungal diseases of turf grasses and various ornamental plants. As a growth regulator, it is used to accelerate natural abscission in citrus fruits to facilitate harvesting. The common name for the substance is cycloheximide. The Chemcial Abstracts Registry (CAS) number is 66-81-9 and the EPA code number is 043401.

In the U.S., The Upjohn Co. is the basic producer of cycloheximide. At present, six products are registered containing cycloheximide as a sole active ingredient. Currently, only three products with fungicidal uses are being marketed.

As a fungicide, cycloheximide is registered for control of certain diseases of turf, hawthorn cedar, California live oak, white pine, non-bearing sour cherries, and various ornamentals. Registered formulations include .027%, 2.1%, 2.26% and 5% active ingredient (A.I.) wettable powders (WP) and a 4.0% A.I. emulsifiable concentrate (EC). Of these, the 2.26% WP and 4.0% EC are no longer marketed and their use is limited to existing stocks.

Approximately 95% of the cycloheximide produced each year is used on turf to control a number of fungal diseases including melting-out, fading-out, dollar spot, rust, and powdery mildew. The primary use is on golf course greens and fairways in the northern states where cool, mist conditions in the spring and fall encourage the growth of dollar spot and Helminthoporiums melting-out.

Application is either by hand sprayers or ground equipment at rates ranging from 0.3 - 1.8 oz. A.I. per acre. Cycloheximide is also sold in combination with Thiram® or polychloronitrobenzene for turf use. Currently, use of cycloheximide on ornamentals and other non-turf sites is minimal.

As a growth regulator, cycloheximide is registered for use in Florida to enhance fruit abscission to aid in the harvest of oranges and other citrus intended for processing. The 4.22% A.I. wettable powder is registered for this use, but also is not currently marketed. According to the label, cycloheximide is applied 4-7 days before harvest. Applications can be made by hand-sprays, by ground sprayer or aerially at rates ranging from 0.3-1.4 oz. A.I. per acre.

At this time, cycloheximide products containing more than 4.0% A.I. are classified as Restricted Use pesticide products and must be applied by a certified applicator.

## C. Regulatory Position

The Agency has considered the scientific data concerning cycloheximide from the open literature, and the data submitted to the Agency by the registrant through May of 1982. Reports in the literature suggest that cycloheximide may be mutagenic and neurotoxic and may inhibit the early and late immune responses. The Agency is concerned about these suggested effects, but at this time does not have sufficient information on these effects, environmental persistence or human exposure to the pesticide to confirm these effects or to conduct an assessment of the risks associated with the registered use patterns of cycloheximide. The Agency, therefore, is requiring through this Standard that data be submitted to characterize the toxicity of cycloheximide, its persistence in the environment, human exposures associated with its use and potential risks to man and the environment. Until the required data are evaluated, the Agency will continue the registration of products containing cycloheximide as the sole active ingredient or in mixtures, but only under the conditions specified here and elsewhere in this Standard. These include:

- The registrants must develop the data specified as data gaps in Chapter III to maintain existing registrations or to obtain new registrations. These data include applicator and dietary exposure information.

- All products containing more than 4.0% cycloheximide active ingredient will continue to be classified as "Restricted Use" pesticide products.

## D. Regulatory Rationale

A review of the available data on cycloheximide shows that much of the information needed to support registration and reregistration of the pesticide are not available, or are inadequate to fully assess risk resulting from its use.

The Agency has the prerogative to not only request information to satisfy the data Guidelines proposed in 1978 and 1980, but also to require additional testing or information under 3(c)(2)(B) of FIFRA. These additional tests may be requested by the Agency if it believes that particular concerns regarding the health or environmental effects of a pesticide must be evaluated.

It is the policy of the Agency not to automatically request guideline information simply because a particular guideline exists. Each element of the guideline is considered individually with regard to the pesticide's uses, exposure and hazard. If the Agency concludes that specific guidelines data are required for a comprehensive Registration Standard, the data will be requested.

Although the data base for cycloheximide is incomplete and deficient, the Agency has concluded that it should continue registration for this product until the data base can be completed for the following reasons:

First, while potential adverse effects are suggested by test data, the effects have not been demonstrated conclusively. The available evidence for these adverse effects is insufficient to justify regulatory action by the Agency.

Second, cycloheximide has been registered for use since 1970. During the period between 1970 and 1979, the Pesticide Incident Monitoring System (PIMS) records show that 8 incidents were reported for cycloheximide of which 7 involved human exposures. Each of these incidents involved applicators and resulted in short-term dermal or eye irritation after exposure. These reports do not provide a basis for regulatory action.

Third, on April 20, 1979, the Registration Division of the Office of Pesticide Programs determined that formulations of cycloheximide containing greater than 4.0% active ingredient should be classified as Restricted Use pesticide products, because of the acute dermal hazard posed to humans and because of the effects that potential residues on treated trees and plants were expected to have on mammalian and avian species. This decision, based on an evaluation of the data available at the time, was to be reevaluated during the Registration Standard development process for cycloheximide. "Restricted Use" classification was considered for cycloheximide in the development of this Standard, but since the information required to make a comprehensive "Restricted Use" classification determination for all cycloheximide products is currently not available, a final decision cannot be made at this time. The Agency will therefore continue the April 1979 decision requiring a "Restricted Use" classification only for formulations of cycloheximide containing greater than 4.0% active ingredient.

Fourth, in accordance with FIFRA, the Agency's policy is not to routinely cancel existing regristration of products or to withhold registration merely for the lack of data (See Sections 3(c)(2)(B) and 3(c)(7) of FIFRA) unless risks are clearly identified. Rather, the publication of this Standard provides a mechanism for identifying data needs; and registration of cycloheximide under this Standard will allow for the upgrading of labels during the period in which the required data are being generated.

## E. Criteria for Registration Under the Standard

To be subject to this Standard, EPA-registered pesticides containing cycloheximide must meet the following conditions:

- o Contain cycloheximide as an active ingredient and have the same use patterns described in this Standard;
- o Bear EPA accepted labeling; and
- o Conform to the acute toxicity limits, product composition, and use requirements stated below.

The applicant for registrationn or reregistration of cycloheximide products subject to this Standard must comply with all terms and conditions described in this Standard, including a commitment to fill data gaps on a time schedule specified by the Agency, and, when applicable, offer to pay compensation to the extent required by Sections 3(c)(1)(D) and 3(c)(2)(D) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended, 7 U.S.C. 136 a(c)(1)(D) and 136 h(c)(2)(D).

## F. Acceptable Ranges and Limits

### 1. Product Composition Standards

To be covered under this Standard, manufacturing-use products must contain cyclo-heximide, 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxethyl] glutarimide, as their sole active ingredient at a minimum of 80%. In addition, these products must not contain impurities other than those in currently registered manufacturing-use products and at no higher concentration than those in currently registered products. Any manufacturing product not meeting these requirements will be considered a new product and will not be automatically registrable under this Standard.

### 2. Acute Toxicity Limits

The Agency will consider registration of manufacturing-use products containing cycloheximide regardless of the toxicity category, provided that the labeling of such products bears appropriate precautionary statements.

### 3. Use Patterns

To be covered under this Standard, manufacturing use products must be labeled to allow for formulation into end-use products that are intended only for one or a combination of the following use patterns:

- o Food/Feed, Nondomestic, Outdoor Use (citrus)
- o Nonfood/Nonfeed, Domstic/Nondomestic, Outdoor use (turf, trees, ornamentals)
- o Nonfood/Nonfeed, Domestic/Nondomestic, Indoor Use (greenhouse)

## G. Required Labeling

All manufacturing-use cycloheximide products must bear appropriate labeling as specified in 40 CFR 161.10. The Agency may, after review of data to be submitted under this Standard, impose additional labeling requirements.

### 1. Manufacturing-Use Products

#### a. Use Pattern Statements

All manufacturing-use products containing cycloheximide must list on the label a statement which provides that the product may be used only in the formulation of products approved by the Agency for one or more of the following use categories:

- o Food/Feed, Nondomestic, Outdoor Use (citrus)
- o Nonfood/Nonfeed, Domestic/Nondomestic, Outdoor use (turf, trees, ornamentals)
- o Nonfood/Nonfeed, Domestic/Nondomestic, Indoor Use (greenhouse)

b. Precautionary Statements

Based on fish and wildlife data currently available, the Environmental Hazard portion of the technical product label must contain the following precautionary statements:

> This product is toxic to fish, birds, and other wildlife. Do not apply directly to water. Do not contaminate water by cleaning of equipment or disposal of wastes.

### 2. End-Use Cycloheximide Products

Although this Standard does not address the registration of end-use products for cycloheximide, revised labeling will be required on end-use products. These requirements will be issued under the Label Improvement Program of the Office of Pesticide Programs' Registration Division. The Agency may, after review of data to be submitted under this Standard, impose additional labeling requirements.

## H. Tolerance Reassessment

The only established tolerance for cycloheximide (Section 180.336 of the Federal Code of Regulations Title 40, Protection of the Environment, Parts 100 to 399) is for residues of 0.1 part per million of the plant growth regulator cycloheximide in or on the raw agricultural commodity citrus fruits. Data submitted by the Upjohn Company indicated that citrus fruits from trees treated at registered use pattern levels do not exceed the 0.1 pm residue tolerance. If toxicology data required by this Standard indicates a greater risk than previously identified from this exposure, this tolerance and use pattern will be reassessed.

## CHAPTER III. SUMMARY OF DATA REQUIREMENTS AND DATA GAPS

Applicants for registration of manufacturing-use cycloheximide products must cite or submit the information identified in the tables in this chapter. Information on which product-specific data requirements are already met is available in the guidance package.

Each data requirement is identified by the section of the Proposed Guidelines which describes the type of data and when the data are required to be submitted (43 FR, 29696 of July 10, 1978; 43 FR, 37336 of August 22, 1978; and 45 FR, 72948 of November 3, 1980). A discussion of why additional data are necessary, or why data normally required are not necessary for this chemical, are explained in footnotes to the tables. The data requirements specified are the minimum that will be required. Areas where additional data may be required as the result of tiered testing are indicated.

Table III.A-1

### Cycloheximide
### Generic Data Requirements:
### Product Chemistry

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B)? If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.61-3 | Product Identity and Disclosure of Ingredients | Yes | All | Technical grade of active ingredient | Yes | 00011561 | No |
| 163.61-8(c)(1) | Color | Yes | All | Technical grade of active ingredient | Yes | 00011561 | No |
| 163.61-8(c)(2) | Odor | Yes | All | Technical grade of active ingredient | No | - | Reserved1/ |
| 163.61-8(c)(3) | Melting Point | Yes | All | Technical grade of active ingredient | Yes | 00012828,00012866<br>00011561,05015199 | No |
| 163.61-8(c)(4) | Solubility | Yes | All | Technical grade of active ingredient | Partially | 00011561,00011581 | Yes/6 mos.2/ |
| 163.61-8(c)(5) | Stability | Yes | All | Technical grade of active ingredient | Yes | 00011630,00012828 | No |
| 163.61-8(c)(6) | Octanol/Water Partition Coefficient | N/A3/ | - | - | - | - | - |
| 163.61-8(c)(7) | Physical State | Yes | All | Technical grade of active ingredient | Yes | 000121192 | No |

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1/ This requirement is being reserved until the Agency can receive and review inhalation studies and the extent of inhalation hazard is determined. This will avoid possible injury to laboratory personnel.

2/ Solubility in g/100 ml or ppm is required in representative polar and non-polar solvents and in water at 20°C.

3/ Octanol/water partition coefficient is only required for non-polar organic compounds, therefore this is not applicable.

Table III.A-1

## Cycloheximide
## Generic Data Requirements: Product Chemistry, Continued

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.61-8(c)(7) | Density or Specific Gravity | Yes | All | Technical grade of active ingredient | No | - | Yes/6 mos. |
| 163.61-8(c)(9) | Boiling Point | N/A[4] | - | - | - | - | - |
| 163.61-8(c)(10) | Vapor Pressure | Yes | All | Technical grade of active ingredient | No | - | Yes/6 mos. |
| 163.61-8(c)(11) | pH | Yes | All | Technical grade of active ingredient | No | - | Yes/6 mos. |

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[4] Boiling point is required only for liquid compounds.

Table III.A-2

## Cycloheximide
## Generic Data Requirements:
## Environmental Fate

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.161-1 | Hydrolysis | Yes | All | Radiolabeled analytical gr. or technical | No | - | Yes/18 mos. |
| 163.161-2 | Photodegradation in water | Yes | Terrestrial non-crop & orchard crop[1]/ | Radiolabeled analytical gr. or technical | No | - | Yes/18 mos. |
| 163.161-3 | Photodegradation in soil | Yes | Outdoor, Food | Radiolabeled analytical gr. or technical | No | - | Yes/18 mos. |
| 163.161-4 | Photodegradation in air | Yes | Outdoor, Food | Radiolabeled analytical gr. or technical | No | - | Reserved[2]/ |
| **Metabolism Studies** | | | | | | | |
| 163.162-1 | Aerobic Soil | Yes | All | Radiolabeled analytical gr. or technical | No | - | Yes/18 mos. |

Table III.A-2

### Cyclohexímide
### Generic Data Requirements:
### Environmental Fate, Continued

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| - | Effects of microbes on pesticides | Yes | All | Radiolabeled analytical gr. or technical | No | - | Reserved[3]/ |
| - | Effects of pesticides on microbes | Yes | All | Radiolabeled analytical gr. or technical | No | - | Reserved[3]/ |
| - | Activated sludge metabolism | Yes | All | Radiolabeled analytical gr. or technical | No | - | Reserved[3]/ |
| 163.163-1 | Leaching | Yes | Outdoor, Non-Food[4]/ | Radiolabeled analytical gr. or technical | Partial | 00011189<br>00011190<br>05001190 | Yes/18 mos. |
| 163.163-2 | Laboratory volatility | Yes | Outdoor, Food | Radiolabeled analytical gr. or technical | No | - | Reserved[2]/ [5]/ |
| 163.163-3 | Field volatility | Yes | Outdoor, Food | Radiolabeled analytical gr. or technical | No | - | Reserved[2]/ [5]/ |

Table III.A-2

## Cycloheximide
## Generic Data Requirements:
## Environmental Fate, Continued

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.164-1 | Field Dissipation-Terrestrial | Yes | All | Representative Formulation | Partial | 00012843 | Yes/18 mos. |
| 163.165-4 | Laboratory Studies on Fish | Yes | All | Radiolabeled analytical gr. or technical | Yes | 00012864<br>00012880 | No[6] |
| Subpart K | Reentry | [7] | All | - | - | - | - |

## Table III.A-2

## Cycloheximide-Registration Standard

## Footnotes to Environmental Fate Data Requirement Chart

(1) Data are required to support terrestrial non-crop and orchard crop uses but not domestic outdoor uses.

(2) Data may be needed to support orchard crop uses if it is found after a case-by-case determination that a significant inhalation exposure to workers exists. The inhalation exposure data are requested on Table A-3.

(3) Specific data requirements and testing protocols needed to generate these data are being developed. No data are required at this time.

(4) Two additional rapid leaching studies are needed on sandy loam, clay or clay loam soil. If suitable (soil/water) relationship values cannot be calculated from soil column or soil thin-layer chromatography data, batch equilibrium (adsorption/desorption) studies will be needed to support domestic outdoor uses.

(5) Data that meet test standards for the field volatility studies will also satisfy requirements for laboratory volatility studies.

(6) Available data indicate that cycloheximide does not accumulate significantly in fish. It is also found to have an octanol/water partition coefficient less than 1000 based on its relatively high water solubility.

(7) After applicator exposure data are received, any requirements for reentry data will be determined on a case-by-case basis.

Table III.A-3

### Cycloheximide
### Generic Data Requirements:
### Toxicological Effects

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.81-1 | Acute Oral (rat) | Yes | All | Technical | No | - | Yes/6 mos. |
| 163.81-2 | Acute Dermal (albino rabbit) | Yes | All | Technical | No | - | Yes/6 mos. |
| 163.81-3 | Acute Inhalation (rat) | Yes | All | Technical | No | - | Yes/6 mos.[1]/ |
| 163.81-4 | Primary Eye Irritation (albino rabbit) | Yes | All | Technical | No | - | Yes/6 mos. |
| 163.81-5 | Primary Dermal Irritation (albino rabbit) | Yes | All | Technical | No | - | Yes/6 mos. |
| 163.81-6 | Dermal Sensitization (guinea pig) | Yes | All | Technical | No | - | Yes/6 mos. |
| 163.82-1 | Subchronic Oral (rat and dog) | Yes | Outdoor, Food | Technical | No | - | Yes/9 mos. |
| 163.82-2 | Subchronic 21-day dermal (rabbit) | Yes | All | Technical | No | - | Reserved[2]/ |
| 163.82-4 | Subchronic inhalation (rat) | Yes | All | Technical | No | - | Reserved[1]/ |
| 163.82-5 | Subchronic neurotoxicity (mammalian) | Yes | All | Technical | No | - | Reserved[3]/ |

Table III.A-3

## Cyclohexinide
## Generic Data Requirements:
## Toxicological Effects

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.83-2 | Chronic feeding (rat) | Yes | Outdoor, Food | Technical | No | - | Reserved[4]/ |
| 163.83-2 | Oncogenicity (rat and mouse) | Yes | Outdoor, Food | Technical | No | - | Reserved[4]/ |
| 163.83-3 | Teratogenicity (two mammalian species) | Yes | All | Technical | No | - | Yes, 9 mos.[5]/ |
| 163.83-4 | Reproduction (one mammalian species) | Yes | Outdoor, Food | Technical | No | - | Reserved[6]/ |
| 163.84-1 thru 4 | Mutagenicity | Yes | All | Technical | No | - | Yes, 24 mos.[7]/ |
| 163.85-1 | Metabolism (one mammalian species) | Yes | Outdoor, Food | Technical | No | - | Reserved[4]/ |
| - | Immunotoxicity (mammalian species) | Yes | Outdoor, Food | Technical | No | - | Yes, 18 mos.[8]/ |
| - | Estimate of Applicator & Dietary Exposure | Yes | All | Representative End-use Product | No | - | Yes, 12 mos.[9]/ |

Table III.A-3 Footnotes

1/ An acute inhalaton test should include an extended observation period (six weeks). The requirement for subchronic inhalation testing is reserved pending analysis of acute inhalation and dietary and applicator exposure data requested on this table.

2/ The subchronic dermal 21-day test is required unless subchronic oral tests are performed, in which case the requirement is reserved pending analysis of acute dermal and exposure data requested on this table. 3/ May be combined with the subchronic oral test.

4/ This test requirement is reserved pending analysis of exposure data requested on this table.

5/ A teratogen may produce significant effects following acute exposure. Since workers may be acutely exposued to significant amounts of this pesticide, two teratogenic studies are required in order to assay what, if any, teratogenic risks/hazards cycloheximide poses to the worker.

6/ The requirement is reserved pending analysis of the revised report of the three generation rat reproduction study.

7/ Six tests are required. These are: gene mutation assay in mammalian cells, an in vitro and an in vivo cytogenetics assay, a Salmonella test to include strains TA 100 and TA 98, and a cytogenetics assay designed to quantify any spindle effects that may be produced by cycloheximide.

8/ This test requirement may be satisfied by analyzing a subset of test animals from the subchronic oral study.

9/ While guideline requirements regarding minimum residue data have been satisfied, the actual dietary exposure is required to fully assess the total exposure to man. As noted earlier, the application rate of cycloheximide is very low. It is possible that, if the actual exposure resulting from the application and dietary consumption of crops treated with cycloheximide were known, the Agency could conclude that the compound does not pose an unreasonable risk to man or the environment. Therefore, the Agency will reserve the right to request all of the toxicology data normally required for a pesticide under Registration Standards review. The Agency will require, instead the minimal toxicology tests, information on exposure to pesticide applicators, and dietary exposure to cycloheximide. Registrants are advised to consult with appropriate Agency scientists before initiating these studies. Upon receipt of the exposure data, the Agency will determine whether the reserved toxicological data will be required.

Table III.A-4

Cycloheximide
Generic Data Requirements:
Residue Chemistry

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| - | Metabolism in plants | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 05013629,05015971<br>05011721,05011721<br>05013502,05013890<br>05016060,00515196<br>05013608,05013617<br>05013928,05013086<br>00012879,00012873<br>00011227,05013510 | No |
| - | Metabolism in Animals | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 00011607 | No |
| - | Analytical Methods | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 00011225,00012877<br>00012873,05013638<br>00011222 | No |

Table III.A-4

# Cycloheximide
## Generic Data Requirements:
## Residue Chemistry, Continued

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| - | Residue Data: Crops | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 00011228,00011229<br>00011230,00011201<br>00012825,00011200<br>00011199,00014434<br>00011198,00011197<br>00012847,00012848<br>00012849,00012850<br>00012846,00012851<br>00012852,00012853<br>00012854,00014438<br>00012860,00012861<br>00012862,00012863<br>00012859,00012858<br>00012857,00012856<br>00012855,00014439<br>00012854,00014435<br>00014436,00014486<br>00011533,00011532<br>00011357,00011538<br>00011534,00011536<br>00011531,00011529<br>00011530,00011535<br>00011528,00012878<br>00011534, | No |

Table III.A-4

## Cycloheximide
## Generic Data Requirements:
## Residue Chemistry, Continued

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| - | Residue Data: Citrus By-Products | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 00011231 | No |
| - | Residue Data: Meat and Milk | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 00011607 | No |
| - | Storage Stability | Yes | Outdoor, Food | Residue of active ingredient and its metabolites which may be of toxicological concern | Yes | 00011226,00012831 | No |

Table III.A-5

### Cycloheximide
### Generic Data Requirements:
### Ecological Effects

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Use Pattern | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B) If So, When Due? |
|---|---|---|---|---|---|---|---|
| 163.71-1 | Avian Single-Dose Oral $LD_{50}$ | Yes | All | Technical | Yes | 00012827 | No |
| 163.71-2 | Avian Dietary $LD_{50}$ | Yes | All | Technical | No | - | Yes/12 mos. |
| 163.71-4 | Avian Reproduction | Yes | All[1] | Technical | No | - | Yes/24 mos. |
| 163.71-5 | Simulated and Actual Field Testing for Mammals and Birds | Yes[2] | All | Technical | No | - | Yes/12 mos. |
| 163.72-1 | Fish Acute $LC_{50}$ | Yes | All | Technical | Yes | 00012895<br>00012894 | No |
| 163.72-2 | Acute Toxicity in Aquatic Invertebrates | Yes | All | Technical | No | - | Yes/12 mos. |
| 163.72-3 | Acute Toxicity in Estuarine and Marine Organisms | Yes | All | Technical | No | - | Yes/24 mos. |
| 163.72-4 | Fish Early Life-Stage/Aquatic Invertibrates Life-cycle | Yes | Outdoor, Non-food | Technical | No | - | Yes/24 mos. |

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[1] Required for uses with multiple applications.

[2] The registrant may substitute a simulated avian feld study, preferably on bobwhite quail, for the two avian dietary tests, which were inconclusive.

Table III.B-1

## Cycloheximide
## Product-Specific Manufacturing-Use Data Requirements:
## Product Chemistry

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B)? If So, When Due? |
|---|---|---|---|---|---|---|
| 163.61-3 | Product Identity and Disclosure of Ingredients | Yes | Each Product | Yes | 00011561 | No |
| 163.61-4 | Description of Manufacturing Process | Yes | Each Product | Yes | 00011192 | No |
| 163.61-5 | Discussion of Formation of Unintentional Ingredients | Yes | Each Product | No | - | Yes/6 mos[1] |
| 163.61-6 | Declaration and Certification of Ingredient Limits | Yes | Each Product | Yes | 00011561 | No |
| 163.61-7 | Product Analytical Methods and Data | Yes | Each Product | Yes | 00012869 | No |

---

[1] In addition to guideline requirements concerning formation of unintentional ingredients, the discussion should specifically address the formation of impurities which occur in quantities up to 20% in the technical grade of the active ingredient. The discussion should also address the potential for formation of nitrosamines during the product of cycloheximide including either an analysis of technical nitrosamine content or a theoretical discussion regarding its formation in the manufacturing or reformulation processes. These additional data are required by 42 FR 51640, where the Agency describes products most likely to be contaminated with nitrosamines, and the proposed nitrosamines policy, 45 FR 42854.

Table III.B-1

## Cycloheximide
## Product-Specific Manufacturing-Use Data Requirements:
## Product Chemistry, Continued

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B)? If So, When Due? |
|---|---|---|---|---|---|---|
| 163.61-8(c)(7) | Physical State | Yes | Each Manufacturing-Use Product | Yes | 00011192 | No |
| 163.61-8(c)(8) | Density or Specific Gravity | Yes | - | No | - | Yes/6 mos. |
| 163.61-8(c)(12) | Storage Stability | Yes | - | Yes | 00011630,00012828<br>00011561,00011192 | Yes/6 mos.[2] |
| 163.61-8(13) | Flammability | Yes | - | No | - | Yes/6 mos. |
| 163.61-8(14) | Oxidizing or Reducing Action | Yes | - | No | - | Yes/6 mos. |
| 163.61-8(15) | Explosiveness | Yes | - | No | - | Yes/6 mos. |
| 163.61-8(16) | Miscibility | N/A[3] | - | - | - | - |
| 163.61-8(17) | Viscosity Coefficient | N/A[4] | - | - | - | - |
| 163.61-8(18) | Corrosion Characteristics | Yes | - | No | - | Yes/6 mos. |

---

[2] The available data are not quantitative as required in the guidelines.

[3] Miscibility data are required only for emulsifiable liquid formulations of manufacturing-use products.

[4] Viscosity data are required only for liquid manufacturing-use products.

Table III.B-2

Cycloheximide

Product-Specific Manufacturing-Use Data Requirements:
Toxicological Effects

| Guidelines Citation | Name of Test | Are Data Required for this Standard? | Composition to be Tested | Does EPA Have Data to Partially or Totally Satisfy this requirement? | Bibliographic Citation (MRID #) | Must Additional Data be Submitted Under FIFRA 3(c)(2)(B)? If So, When Due? |
|---|---|---|---|---|---|---|
| 163.81-1 | Acute Oral (Rat) | Yes | Manufacturing-Use Product | No | | Yes/6 mos. |
| 163.81-2 | Acute Dermal (albino rabbit) | Yes | Manufacturing-Use Product | No | | Yes/6 mos. |
| 163.81-4 | Primary Eye Irritation (albino rabbit) | Yes | Manufacturing-Use Product | No | | Yes/6 mos. |
| 163.81-5 | Primary Dermal Irritation (albino rabbit) | Yes | Manufacturing-Use Product | No | | Yes/6 mos. |

## CHAPTER IV. PRODUCT CHEMISTRY

### A. Introduction

FIFRA 3(c)(2)(A) requires the Agency to establish guidelines for registering pesticides in the United States. Subpart D of the guidelines explains what product chemistry data must be submitted by the registrant in order for the Agency to characterize the product, assess the environmental and health effects associated with its use, and otherwise evaluate an application for registration.

To evaluate product composition, the Agency requires: 1) the submission of a detailed manufacturing process for pesticides including data on the composition of starting and intermediate materials; 2) a discussion of the presence in a product of unintentional ingredients formed during, or subsequent to, manufacture; 3) declaration of the upper and lower limits for each active and intentionally added inert ingredient, and the upper limit for each impurity; 4) certification that ingredient limits will be maintained for all quantities of the product sold or distributed in commerce; and 5) analytical methods, and data obtained by these methods, for each active ingredient and identifiable impurity at or in excess of 0.1% of the product weight. The Agency may require methods and data for impurites below 0.1% of the product weight when highly toxic impurities are present.

The Agency also requires data on the physical and chemical properties of the pesticide. For example, data are needed concerning the identity and physical state of the active ingredient (e.g., melting and boiling points, vapor pressure, and solubility). Data are also required on those properties of the formulated product that are clearly related to necessary labeling cautions (e.g., flammability, corrosion characteristics, and storage stability).

To assist applicants in meeting these requirements and to accelerate the Agency's review and evaluation of the submitted data, the guidelines include criteria for acceptance testing of products and an appendix with references to literature sources containing appropriate testing protocols.

These guidelines request that product chemistry data be submitted on both the technical and the manufacturing-use product. In the case of cycloheximide, the technical material and the manufacturing-use product are the same, so registrants may submit data on either to satisfy the data requirements.

### B. Chemical Identity

In the United States, "Cycloheximide" is the acceptable common name adopted by the National Standards Institute for

3[2-(3,5-dimethyl-2-oxocyclohexyl)-2hydroxethyl] glutarimid, which has the follow chemical structure:

Identifying characteristics of cycloheximide include: molecular formual, $C_{15}H_{23}NO_4$; molecular weight, 281.4.

Trade names for cycloheximide include Acti-Dione (a fungicide), Acti-Dione TGF, Acti-Dione RZ, Acti-Dione BR Concentrate, Acti-Dione PM (flower fungicide). The Chemical Abstracts Registry (CAS) code number is 66-81-9 and other identification numbers are ENT-15541 and U-4527. Product names include Acti-Acid (citrus abscission agent).

## C. Manufacturing Process

A detailed description of the manufacturing process is required because the method and route by which a pesticide is synthesized determines the nature and amount of potentially toxic impurities.

Technical cycloheximide is biosynthesized by a fermentation process utilizing various streptomycin-producing strains of Streptomyces grieseus (Ford and Leach, Journal of the American Chemical Society, 70, 1223, 1948). A search of the open literature reveals a series of U.S. and foreign patents for the manufacture, separation, purification and use of cycloheximide as a pesticide (MRID #05013808, #05013809, #05013831, #05013834).

In the U.S., the TUCO Division of the Upjohn Co. is the basic producer of cycloheximide (Farm Chemicals Handbook, 1981). TUCO has submitted product chemistry data (see MRID #00011192) that are sufficient to meet the Agency's requirements for a description of the manufacturing process. Since the manufacturing process is considered to be confidential business information, discussions about the specific procedures, equipment, and conditions required for commercial manufacture are not contained in the body of this Standard. Details of the manufacturing procedure are discussed in Appendix A which contains confidential information protected under Section 10 of FIFRA.

## D. Formation of Unintentional Ingredients

The proposed product chemistry guidelines (July 1978) require that the registration of each pesticide product be supported by a discussion concerning the formation of impurities and intentionally added inert ingredients that may be present in the pesticide product. The discussion should address impurities from equipment, packaging, reactions between ingredients, degradation of the chemicals, or those impurities introduced because of processing variables, purification or quality control measures, or any other relevant factors. In addition to these items for cycloheximide, the discussion should specifically address the formation of the "manufacturing impurities" which occur in quantities up to 20% in the technical grade of the the active ingredient. The discussion should also address the potential for formation of nitrosamines during the production of cycloheximide, including either an analysis of the actual nitrosamine content or a theoretical discussion regarding their formation in the manufacturing or reformulation processes. Required information regarding the formation of unintentional ingredients or impurities is not currently available and therefore constitutes a data gap.

## E. Declaration and Certification of Ingredient Limits

### 1. Technical Grade Cycloheximide

Technical grade cycloheximide (which is identical to the manufacturing-use cycloheximide) contains a minimum of 80% 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide as the active ingredient. When cycloheximide is manufactured according to the process previously described it usually contains a minimum of 80.0 percent of the active ingredient, but it may contain more. The upper range is known but is considered to be confidential (contained in Appendix A), so the range of the upper and lower limits for the active ingredient are known.

A maximum of 20% of the technical product consists of substances referred to collectively as "manufacturing impurities." The upper limit for these collective impurities has been set and certified at 20%, but upper limits for the individual impurities have not been set or certified. This constitutes a data gap.

## F. Product Analytical Methods and Data

A method for analyzing cycloheximide in both technical and manufacturing-use products has been submitted to the Agency. The procedure for analysis of cycloheximide is called cylinder-plate assay. It involves measuring the zone of inhibited growth of Saccharomyces cerevisiae (VC 1606) in the presence of cycloheximide. By comparing the resulting zone of inhibited growth with standard plate inhibition zones, the concentration of cycloheximide can be calculated (MRID #00012869). While alternate methods of analysis for cycloheximide are reported in the scientific literature, the cylinder-plate method is considered to be adequate to satisfy the guideline requirements.

## G. Physical/Chemical Properties

The physical/chemical properties of technical grade cycloheximide depend on the ingredients and the strain of *Streptomyces griseus* used to produce cycloheximide, and both of these are primarily a function of the manufacturing process. The physical and chemical properties of crystalline cycloheximide that have either been subnmitted by TUCO or are found in the open literature and generally accepted are as follows:

Color: Colorless (MRID #00011561)

Melting Point: 119.5 - 121°C (Merck Index, 9th Ed., MRID #00012828, #00012866, #00011561)
115 - 117°C (MRID #05015199)
115.5 - 119°C (Condensed Chemical Dictionary, 8th Ed.)

Solubility:

Soluble in water (MRID #0011561), 2.1 g/100 ml at 2°C (Merck Index, 9th Edition ) in common organic solvents except saturated hydrocarbons (MRID #0011561), and in amyl acetate = 7 g/100 ml at 2#C (Merck Index, 1981, and MRID #0001158).

Stability:

Technical grade cycloheximide is most stable as a dry crystalline powder or dissolved in an inert anhydrous solvent (MRID #00011630). In the crystalline form, it is relatively thermostable (MRID 00012828, #00011561).

When exposed to water, cycloheximide becomes very unstable. The maximum stability in aqueous solution is at pH 4-5 and rapid decomposition occurs above pH 7. Cycloheximide is unstable to alkaline conditions with hydrolysis occuring (MRID #00011192).

The Merck Index, 9th Ed., states that cycloheximide is relatively heat stable and acid stable, being completely destroyed by boiling in aqueous solution at pH 7 for 1 hour, but with no loss of activity after 15 minutes of boiling. It further reports that cycloheximide is rapidly inactivated at room temperature by dilute alkali and forms a volatile, fragrant ketone, 2,4-dimethylcyclohexanone.

Physical State: Crystalline Plates (MRID #00011192).

Boiling Point: (Not required for solids.)

Storage Stability: Unstable in almost any form except as a dry crystalline solid or in an inert anhydrous solvent (MRID #00011630, #000128228, #00011561 and #00011192).

Miscibility: Not applicable.
Viscosity: Not applicable.

Dielectric Breakdown Voltage: Not applicable. (Current use does not include applications around power lines and transformer stations, so no data are required. If patterns were modified to include those uses, data for this property would be required.)

## H. Data Gaps

The physical and chemical properties on which data are unavailable and which constitute data gaps are as follows:

Odor: Data required but reserved until subchronic inhalation studies can be completed to determine extent of suspected inhalation hazard.

Solubility: Solubility data for representative polar and nonpolar solvents and for distilled water at 20°C are required in g/100 ml of solvent at 20°C (68°F) or in terms such as ppm (mg/kg). Accordingly, available solubility data are incomplete and constitute a data gap.

Density or Specific Gravity: Data are needed.

Vapor Pressure: Data are needed.

pH: Data are needed

Storage Stability: The proposed Guidelines require quantitative analysis of the manufacturing-use product to determine storage stability. The available storage stability data do not meet this requirement.

Flammability: Data are needed.

Oxidizing or Reducing Action: Data are needed.

Explosiveness: Data are needed.

Corrosion Characteristics: Data are needed.

End-Use Products: This Registration Standard does not pertain to cycloheximide end-use products. The product chemistry data requirements, if any, for end-use products are determined at the time of (re)registration.

In accordance with the proposed product chemistry guidelines, additional data and information are required on the presence and formation of impurities during the manufacture of cycloheximide and the declaration and certification of ingredient limits in the technical and/or manufacturing-use product. Physical/chemical data required for the technical grade of the active ingredient are odor, solubility, density or specific gravity, vapor pressure and pH. Where it is also the manufacturing-use product, additional information on storage stability, flammability, oxidizing or reducing action, explosiveness and corrosive characteristics is also required.

## CHAPTER V: ENVIRONMENTAL FATE

### A. Use Summary

At present, six products are registered containing cycloheximide as a sole active ingredient.

As a fungicide, cycloheximide is registered for control of certain diseases of turf, cedar, California live oak, white pine, non-bearing sour cherries, and various ornamentals. Registered formulations include 0.027%, 2.1% 2.26% and 5% A.I. wettable powders (WP) and a 4.0% A.I. emulsifiable concentrate (EC). Of these, the 2.26% WP and 4.0% EC are no longer marketed and their use is limited to existing stocks.

Approximately 95% of the cycloheximide produced each year is used on turf to control a number of fungal diseases including melting-out, fading-out, dollar spot, rust, and powdery mildew. Primary use is on golf course greens and fairways in the northern states where cool, moist conditions in the spring and fall encourage the growth of dollar spot and Helminthoporiums melting-out. Application is either by hand sprayers or ground equipment at rates ranging from 0.3-1.8 oz. active ingredient per acre. Cycloheximide is also sold in combination with Thiram® or polychloronitrobenzene for turf use. Current use of cycloheximide on ornamentals and other non-turf sites is minimal.

As a growth regulator, cycloheximide is registered for use in Florida to stimulate abscission to aid in the harvest of oranges and other citrus fruits that are intended for processing. The 4.22% A.I. wettable powder is registered for this use, but is not currently marketed. According to the label directions, cycloheximide is applied 4-7 days before harvest. Applications can be made by hand sprays, by ground sprayer or aerially at rates ranging from 0.3-1.4 oz. a.i. per acre.

At this time, cycloheximide products containing more than 4.0% A.I. are classified as restricted use only and must be applied by a certified applicator. (See discussion under Chapter II Regulatory Rationale.)

### B. Environmental Fate Profile

Much of the data submitted and reviewed to provide environmental fate information are in areas that are either not of direct concern to the Agency or were generated in the laboratory under conditions that do not reflect actual environmental conditions. These data therefore are of limited use in characterizing the environmental fate of cycloheximide. Useful information has been extracted from the data base and is presented in the following discussion; however, data requirements have not been fully satisfied in many cases.

### 1. Hydrolysis

In tests, cycloheximide has been dehydrated in solution to yield trans anhydrocycloheximide, which is rehydrated to yield a-epicycloheximide and/or a-epi-Naramycin B (MRID #00011594). Dehydration is very rapid in alkaline and highly acidic solutions (MRID #00011595). The slowest rate of dehydration occurs at pH 4.4, and the rate increases with increasing temperature. These tests were conducted under laboratory conditions instead of the environmental conditions required by the guidelines. As a result, the precise estimates of reaction rates and product values under typical environmental conditions are not available. Based on the laboratory data which are available, maximum yields of these products are expected to be >10% of the applied cycloheximide. It appears that a minor transformation product (<10%) may be derived from the isomerization of trans anhydrocycloheximide to yield the cis isomer and then rehydrated to give isocycloheximide, but this has not been definitively shown (MRID #00011594).

The glutarimide ring of cycloheximide, its isomers, and anhydrocycloheximide are readily hydrolyzed to yield the monocarboxylic acid amide (MRID #00011594 and #00011595). The reaction occurs instantly in highly alkaline solutions, and the rate decreases with decreasing pH. Further hydrolysis yields ammonia and the dicarboxylic acid. The latter reaction occurs readily in acidic solutions but is rate-limiting in alkaline solutions.

At 20-30°C, hydrolysis reaches 10% completion in 2-3 and 7-11 days at pH 9 and 7, respectively.

The temperature at which the tests (alkaline conditions) were conducted was not as recommended by registration guidelines, analysis of the data show that sufficient information on hydrolysis under alkaline conditions has not been provided. Testing under acid conditions (pH 3-5.5) was conducted at temperatures (50-80°) which do not represent those found under actual use conditions. While information is available concerning the hydrolysis of cycloheximide, it is not adequate, when considered in toto, to meet the Agency's needs. Therefore, a hydrolysis study is needed.

### 2. Soil Metabolism

Available studies indicate that cycloheximide dissipates rapidly from various soils and that dissipation rate is affected by temperature and soil type. Cycloheximide, at 0.13-10 ppm, had a half-life range of 1.5-3 days in loam soil and in an unidentified soil at 24° and 28°C, respectively (MRID #005013612), in a sandy soil outdoors in a container in Florida during January (MRID #00012843), and in a mixture of three soils at 35°C (MRID #00011196 and #00012845). When $^{14}C$ cycloheximide was used in the soil mixture, extractable $^{14}C$ had a half-life of about 2 days (MRID #00012845). More than 80% of the applied $^{14}C$ volatilized during 9 days of incubation, and <15% was unextractable. Microorganisms appear to contribute to degradation of cycloheximide in soil, as determined by comparison of sterile and nonsterile soil samples (MRID #005013612 and #00012845) but problems with this type of testing make this difficult to confirm. No data were available from any of these tests on the degradation products formed in soil.

These tests were not conducted in a manner which could determine whether the cycloheximide was being degraded or volatilized, and they were not designed to indicate which degradation products were formed in the soils. Both of these conditions are specified under the guidelines. As such, the requirements for soil metabolism are not satisfied and this constitutes a data gap.

### 3. Microbial Effects

Available data indicate that the growth of most bacteria in soil should not be affected by cyclohedimide at low concentrations ($\leq$1 ppm) (MRID #05019056). Cycloheximide, at 4,000 ppm, has been shown to inhibit soil respiration for 6 hours (MRID #05016745), but no data are available for lower concentrations and longer periods. The growth of some bluegreen algae was inhibited by cycloheximide at $\geq$50 ppm (MRID #05021783 and 05012934) and *in vitro* nitrogen fixation was markedly inhibited by cycloheximide at $\geq$50 ppm (Kapoor and Sharma, #05021783). Cycloheximide also inhibited nodule formation in the symbiotic association between *Rhizobiumtrifolii* and clover (Robinson, #05013679). Consistent with its major use, cycloheximide is toxic to fungi. Based on the available data (MRID #05019056, 05013934, 05014316, 05013615, 05016063, 05021327, 05013498, 05013346 and 005013612) it is expected that cycloheximide in soil at $\geq$1 ppm will markedly inhibit fungal growth. Cycloheximide, at 0.7 ppm, induced the elimination of extrachromosomal genes in *Saccharomyces cerevisiae* (MRID #05016140). Cycloheximide also is toxic to other eucaryotic organisms. At 30 ppm, cycloheximide inhibited respiration by the protozoan *Tetrahymena pyriformis* (MRID #05017110) and rhizoid formation by the green alga *Boergesenia forbesii* (MRID #05021055). Specific data requirements and testing protocols needed to generate microbial effects are being developed. No additional data are required at this time.

### 4. Leaching

In the tests submitted to investigate leaching properties, $^{14}C$ cycloheximide and its residues were very mobile in sand. In one rapid leaching study, over 95% of the applied $^{14}C$ leached through a 12-inch column eluted with the equivalent of 20 acre-inches of water (MRID #00011189). Cycloheximide comprised 64% of the $^{14}C$ residues. When residues were aged 30 days (MRID #00011190), 52% of the applied $^{14}C$ was leached, 15% remained in the soil, and 27% had dissipated. In this study, cycloheximide was not detectable in the leachate. In a soil thin-layer chromatography study (MRID #05001190), cycloheximide was mobile to very mobile ($R_f$, 0.89) in a silty clay loam soil.

The guidelines specify that leaching studies be conducted on four soil types, since these tests were on two soil types the requirements are not satisfied. Two additional rapid leaching studies are needed on sandy loam, clay or clay loam soil.

### 5. Field Dissipation

When tested, cycloheximide dissipated rapidly under field conditions (MRID #00012843) with a half-life of 2 days in sandy soil treated with 4.23% A.I. WP at 0.19 lb A.I./A. These tests partially fulfill the guideline requirements but field data under actual use conditions are needed to support all outdoor terrestrial uses including citrus (orchard crop), terrestrial non-crop (turf) and domestic outdoor uses (ornamentals, turf). Identification and quantification of degradation products is also necessary to meet the data requirements.

### 6. Bioaccumulation in Fish

Available tests indicate that cycloheximide does not accumulate in bluegills. For fish exposed to two doses of $^{14}C$ cycloheximide at 0.09 ppm, the maximum bioaccumulation factor for $^{14}C$ residues was <10 during a one-month exposure period (MRID #00012864 and #00012880). Cycloheximide is relatively soluble in water (2.1% at 2°C, Merck Index, 9th ed., 1976) and therefore should have a low octanol/water partition coefficient. Cycloheximide had a half-life of about 4-7 days in nonsterile water typical of natural systems (MRID #00012864 and #00012880; #00012843). The information on bioaccumulation in fish is adequate to satisfy the data requirements.

### 7. Summary of Accepted Environmental Fate Information

Available data indicate that cycloheximide has a half-life in soil of about a week or less. The identity of products formed in soil are not known from the available data, but loss of products or parent compound by volatilization is indicated. Cycloheximide will be toxic to fungal and protozoan populations immediately following application, but long-term effects are not expected, due to the rapid dissipation rate. Products formed in water are: trans anhydrocycloheximide; cycloheximide isomer(s) that is probably a-epi-Naramycin B and/or a-epicycloheximide; monocarboxylic acid amides and dicarboxylic acids of cycloheximide, its isomers, anhydrocycloheximide; and ammonia. Cycloheximide and its residues appear to readily leach in sandy soil, and it is mobile to very mobile in silty clay loam soil. If cycloheximide enters an aquatic system, it will be rapidly transformed, and residues are not expected to accumulate in aquatic nontarget organisms.

## C. Exposure Profile

Adequate data are not available to assess the potential for exposure of humans and wildlife to cycloheximide. Based on the uses of cycloheximide, there is a potential for groundwater contamination. From the information available, cycloheximide leached readily in sand (MRID #00011189) and indicated high mobility in silty clay loam soils (MRID #05001190), it was transformed in water at rates depending on pH and temperature (MRID #00011594 and #00011595) and available data indicate that the parent compound has a half-life of <5 days in soil (MRID #005013612, #00011196 and #00012843). Cycloheximide was not detectable in groundwater (<20 cm from the surface of sandy soil) for 5 days following treatment with 4.22% A.I. WP and irrigation with 2 inches of water (MRID #00012843).

However, data are not available on the nature of the degradation products formed in the soil. Radiolabeled products of $^{14}C$ cycloheximide aged in sandy soil readily leached (MRID #00011190). This suggests there may be a problem of cycloheximide getting into ground water, but data are needed on the nature of these products to fully assess the potential for exposure through ground water contamination.

Adequate data are not available to assess respiratory exposure to cycloheximide. When $^{14}C$ cycloheximide was aged in sandy soil, 27% of the applied $^{14}C$ appeared to be volatilized after 30 days (MRID #00011190). Similarly, more than 80% of the applied $^{14}C$ appeared to be volatilized during 9 days of incubation of $^{14}C$ cycloheximide in a mixture of three soils at 35°C (MRID #00012845). But the process is not understood, so it is not clear whether the cycloheximide was volatilizing or degrading. Additional information is necessary so that the Agency can assess respiratory exposure.

Human exposure to cycloheximide during mixing and application also cannot be assessed. Respiratory exposure resulting from "puff-back" when the products are opened could be minimized through the use of respirators. Dermal and ocular exposure resulting from splashing during mixing or application, could be minimized by protective clothing and goggles or face shields.

The use patterns of cycloheximide products are characterized by relatively low dosage rates and preclude significant quantities of cycloheximide from entering aquatic systms via runoff from treated areas. Cycloheximide is soluble in water (2.1% at 2°C; Merck Index, 1976) and should have a low octanol/water partition coefficient. Cycloheximide is transformed in water (MRID #00011594 and #00011595; MRID #00012843), and cycloheximide and its residues do not accumulate in bluegill sunfish (Petzold and Chapman, #00012864 and #00012880). Therefore, exposure of humans to cycloheximide via ingestion of contaminated fish is not expected.

Data are not available to assess the potential for dermal and inhalation exposure of golfers, spectators, or groundskeepers following turf applications, for workers entering citrus orchards sprayed with cycloheximide, or for those living or working around treated lawns, trees, or ornamental plants. Wildlife may be exposed to cycloheximide through contact with treated turf, foliage plants, or soil residues.

### D. Summary of Major Data Gaps

The major environmental fate data gaps for cycloheximide are: hydrolysis studies; photodegradation studies in water, on soil, and in air; aerobic soil metabolism studies; two rapid leaching studies in either sandy loam, clay, or clay loam soil, laboratory and field volatility studies; and terrestrial field dissipation studies. Reentry data may be required as determined by further review and the request for those data is reserved. Requirements will vary with different use patterns.

## CHAPTER VI: TOXICOLOGY

### A. Introduction

While studies have been conducted on cycloheximide to evaluate various toxicological characteristics, only a few of those studies are judged to be valid by the Agency. None of the data provided satisfied any guideline requirement. As a result, little information is available which is useful to the Agency in characterizing the toxicity of cycloheximide and extensive testing is necessary to provide the information required. In those cases where tests conducted with cycloheximide suggest that it may cause adverse effects (like immune response inhibition) the Agency is requiring clarification of the studies to confirm and quantify those effects so that potential hazards can be thoroughly evaluated.

### B. Toxicology Profile

Insert A (attached on the following pages) takes the place of this section.

# INSERT A

## TOPICAL DISCUSSIONS

## CYCLOHEXIMIDE

### A. Acute Oral Toxicity (163.81-1)

The minimum data requirement for testing acute oral toxicity (LD50) is one test on the technical chemical and on each manufacturing-use and formulated product, preferably using the laboratory rat.

Swoap (1954) reported cycloheximide acute oral toxicities (LD50 in mg/kg) in the following species:

| Mice | Rats | Guinea Pigs | Rabbits | Dogs |
|---|---|---|---|---|
| 133 | 1.75 | 65 | 6.5 | 65 |

The registrant-submitted experimental records were generally not legible and in most cases the numbers of animals and the times of death or survival cannot be determined. Since cycloheximide has been shown to product delayed death after an acute dose (Kimishima, K. and T. Kado, 1959) an extended observation period is needed.

Thirty-three rats were tested, however no females were used. This is especially important since R. S. Verbin, *et*. *al*. (1971) showed that female Wistar rats were more susceptible to an i.p. dose of cycloheximide than were male rats. These studies have been classified as supplementary and not sufficient to satisfy this data requirement.

### References:

Swoap, O. F. (1954). UpJohn Company Animal Toxicity Reports. Unpublished study received April 16, 1970, under 060880; CDL: 091512-C (00011582/00011212).

Kimishima, K.; and T. Kado, (1979) Sozoku Kihizae Naramcin yakuri, Yonago Igaku Zasshi. 10 (2): 482-485. (05016756/05021072).

Verbin, R. S.; Longnecker, D. S.; Liang, A.; Farber, E. Some observations on the acute histopathologic effects of cycloheximide *in vivo.* Am. J. of Pathology 62 (1): 111-125. (05015404).

## B. Acute Dermal Toxicity (163.81-2)

The minimum data requirement for testing acute dermal toxicity (LD50) is one test on the technical chemical and on each manufacturing use and formulated product, preferably using the albino rabbit.

The acute dermal LD50 of cycloheximide was reported to be 53.2 mg/kg in 12 albino rabbits (3 groups of 4 rabbits [2 males and 2 females] each) (Goyings, L. S., and B. S. Kaxzkofsky, 1968). The study has been classified as invalid since the posttreatment observation was limited to 48 hours, no record was made of systemic toxicity signs or the cause of death, and the skin lesions were not graded.

This study is not sufficient to satisfy the acute dermal toxicity data requirement.

References:

Goyings, L. S.; Kaczkofsky, B. S. (1968). The Acute Dermal LD50 of Acti-Dionep® (Technical Material) in Rabbits. Unpublished study received August 25, 1979, under 1023-EX-27; submitted by Upjohn Co., Kalamazo, Mich., CDL: 210036-D (00011213).

## C. Acute Inhalation Toxicity (163.81-3)

The minimum data requirement for testing acute inhalation toxicity ($LC_{50}$) is on the technical chemical and on each manufacturing-use and formulated product, preferably using the laboratory rat.

Ten rats were exposed to the dust aerosol of cycloheximide under dynamic conditions (nominal concentration of 2.6 mg/liter of air) for one hour (Glenn, M. W. and L. D. Moe, 1968). The animals were observed for 14 days before necropsy. Three rats died on the third day after treatment, one on day six and one on the thirteenth day after treatment. This test has been classified as invalid since only one dose level was tested and at least three are required to establish an $LC_{50}$, no autopsy data were submitted, particle size data were not submitted, the air in the chamber was not sampled for actual dust concentration, and the numbers of deaths stated in the summary and in the actual data do not agree.

The requirement for an acute inhalation toxicity test has not been satisfied; however, this test may be replaced by a subchronic inhalation test. If an acute test is performed, the observation period should be extended. H. J. Horn (1956) stated that the contract laboratory for the study cited above was not able to establish an $LC_{50}$ with an acute dose because of the "marked incidence of delayed deaths." He furthermore stated that "this compound has a most unusual and insidious onset of toxicity.... the only adequate method of evaluation of the inhalation hazard from this compound would be to conduct chronic inhalation exposures." In view of these findings a subchronic inhalation toxicity test will be acceptable in place of an acute inhalation toxicity test.

References:

Glenn, M. W.; Moe, L. D. (1968). One-Hour Inhalation Exposure of Rats to Dust Aerosol of Cycloheximide. (Unpublished Study received August 25, 1969, under 1023-EX-27; submitted by UpJohn Co., Kalamazoo, Mich.; CDL: 210035-F.) (00012829)

Horn, H. J. (1956), letter from Hazelton Laboratories, Falls Church, Va. to M. J. VanderBrook, the Upjohn Company, Kalamazoo, Mich. (000011234).

D. Primary Eye Irritation (163.81-4)

The minimum data requirement for primary eye irritation is one test on each manufacturing-use product and each formulated product, preferably using the albino rabbit.

This test may be waived upon submission of data demonstrating that the test substance has a pH of 1-3 or 12-4; for regulatory purposes, a test substance with a pH of 1-3 or 12-14 will be considered corrosive to the eye.

No data on eye irritation are available. Cycloheximide may well be found to be a severe eye irritant since it was found to be a moderate to severe skin irritant (J. C. Murphy, et al., 1977).

References:

Murphy, J. C.; Skierkowski, P.; Watson, E. S.; Folk, R. M.; Litterst, C. L.; Davis, R. D.; Guarino, A. M. (1977). Cutaneous Irritation in the Topical Application of Cycloheximide NSC-185 to New Zealand White Rabbits. Bethesda, Md.: National Institutes of Health, National Cancer Institute. (Available from: NTIS, Springfield, VA.; PB-414.) (04014425)

E. Primary Dermal Irritation (163.81-5)

The minimum data requirement for primary dermal irritation is one test on each manufacturing-use product and each formulated product, preferably using the albino rabbit.

This test may be waived upon submission of data demonstrating that the test substance has a pH of 1-3 or 12-14. For regulatory purposes, a test with a pH of 1-3 or 12-14 will be considered corrosive to the skin.

Cycloheximide was reported to be a moderate to severe skin irritant in 6 (3 male and 3 female) New Zealand white rabbits at doses up to 9% cycloheximide (J. C. Murphy, et al., 1977). The Draize scores were as follows:

| | | | | |
|---|---|---|---|---|
| Cycloheximide concentration: | 0 | 1% | 3% | 9% |
| Draize score: | 0 | 4.3 | 3.8 | 4.1 |

The study is classified as supplementary since it did not test undiluted technical cycloheximide. It indicated that cycloheximide is a skin irritant, but did not establish the degree of skin irritation that would be caused by cycloheximide technical.

This study is not sufficient to satisfy the primary dermal irritation requirement.

Murphy J. C.; Skierkowski, P.; Watson, E. S.; Folk, R. M.; Litterst, C. L.; Davis, R. D.; Guarino, A. M. (1977). Cutaneous Irritation in the Topical Application of Cycloheximide NSC-185 to New Zealand White Rabbits, Bethesda, Md.: National Institutes of Health, National Cancer Institute. (Available from: NTIS, Springfield, Va.; PB-414.) (05014425)

## F. Dermal Sensitization (163.81-6)

The minimum data requirement for dermal sensitization is an intradermal test for each manufacturing-use product and each formulated product, preferably using the guinea pig.

No data are available on dermal sensitization. Since some uses may result in repeated skin contact, a test is required.

## G. Acute Delayed Neurotoxicity (163.81-7)

The minimum data requirement for acute delayed neurotoxicity is one test for the technical chemical, using the adult hen.

An acute delayed neurotoxicity test is required if the active ingredient, or any of its metabolites, degradation products, or impurities cause esterase depression or are structurally related to a substance that induces delayed neurotoxicity.

No data are available on acute delayed neurotoxicity. An acute delayed neurotoxicity test is not required since cycloheximide does not cause cholinesterase inhibition.

## H. Subchronic Oral Toxicity (163.82-1)

The minimum data requirement for subchronic oral toxicity is one test for the technical chemical in two mammalian species, preferably using the rat and dog.

A subchronic oral toxicity test is required if pesticidal use requires a tolerance or an exemption from a tolerance, requires the issuance of a food additive regulation or is likely to result in repeated human exposure through the oral route.

Three studies were reviewed.

Six monkeys (2 control, 2 at 4 mg/kg, and 2 at 8 mg/kg level) were administered cycloheximide by oral intubation for about 4 weeks (Latven, A. R., 1955). Toxicity (reported only at the high-dose) was characterized by depression, diarrhea, and weakness. The two monkeys at the high-dose level were sacrificed at the sixth and seventh day of treatment.

Histopathology revealed at 4 and 8 mg/kg, generalized degenerative changes (such as cloudy swelling and hydrapic degeneration) in the liver, kidney, stomach, and pancreas, accompanied by a general inflamatory process involving parts of the intestinal tract.

This study was considered valid as a dose range finding experiment; however, it is supplementray as a subchronic test since there were only six animals, only two dose levels, of which the high-dose died at 6 and 7 days respectively, and it was terminated after 26 days.

A second monkey study was conducted using five groups of three Rhesus macaque monkeys (Latven, A. R., 1955) in which they received 0, 0.1, 1, 5, and 10 ppm cycloheximide in the diet for one year. The monkeys were in poor health, 4 died prior to start of experiment and 3 in the first month. Four monkeys were replaced after the start of the experiment, two controls and a high-dose monkey at day 21, and a low dose monkey at day 147. One high-dose monkey died of unknown causes at day 151.

One of two monkeys at 10 ppm showed a mild but progressive anemia throughout the study. Programs were normal with some (degree unspecified) albuminuria at 5, 1 and 0.1 ppm. Terminal organ weights were variable but within normal limits, except that one monkey at 10 ppm had a lighter than normal liver while the other monkey's liver was heavier. Medullary congestion of the kidneys was noted at the two highest levels at gross autopsy. Histopathology found numerous tissue adhesions. These adhesions in the abdominal cavity attest to ongoing and past infections in the monkeys made worse by malnutrition at the time the animals were received. All animals including the controls were found to have glomerulonephritis or interstitial nephritis, or both, with the exception of one monkey in the high-dose group. There appears to be a dose related effect of cycloheximide on central nervous system tissue involving the covering of the brain, pia, arachnoid and dura mater, proliferation of ependymal cells with involvement of the choroid plexus.

All male monkeys had immature testes. One monkey at 5 ppm had fibrosis and slight lyphocytic infiltration of the prostate, severe fibrosis and very thick tunica albuginea of the testes. The males at 10 ppm had immature testes with degenerating cells surrounding portions of rete.

This study is classified as invalid since the animals were in poor physical condition. Monkeys were reported to have tuberculosis and other nonspecific infections. Histopathology confirms internal organ adhesions were extensive, further substantiating the presence of longstanding diseases. Furthermore, not all animals were sexually mature. There was a suggestion of adverse effects on the testes which could not be confirmed.

A third study used 100 male rats. Four groups of 10 rats were fed 1, 2, 5, and 10 ppm cycloheximide by oral gavage. Three groups of 20 rats each were fed only mash diet, liquid diet ad lib, and liquid diet by oral gavage. All the rats at 5 and 10 ppm died shortly after the beginning of the experiment and two additional groups of 10 rats were added and fed at 5 and 10 ppm cycloheximide. These died before the 17-day period. Sixty percent of the animals at 2 ppm died either at or before the fourth month (with enlarged yellowish livers, swollen prostates, atrophied seminal vesicles, testicles, and thymus and partial paralysis of rear limbs (four of ten rats). At 1 ppm, one rat of ten was paralyzed.

At necropsy in the control groups, 46 of 60, and at 1 and 2 ppm, 11 of 20 rats had pneumonia. Yellow livers were observed in 9 of 10 rats at 1 ppm, 8 of 10 at 2 ppm and 0 of 60 in the controls. Enlarged thyroids were reported in 4 rats at 1 ppm and 2 at ppm. Atrophied testes were seen in 7 rats at 2 ppm. Hypertrophied prostates were found in 1 rat at 1 ppm and 4 rats at 2 ppm. One rat at 2 ppm had a small prostate.

This study is classified as supplementary since there was widespread pnemonia in both the controls and treated groups, there were too few animals in the treated groups, and only male rats were used.

These studies are not sufficient to satisfy the requirement for subchronic oral toxicity tests.

References:

Conner, N. D. (1956) Chronic Toxicity in the Rat 1 Year Forced Feeding Study, Submitted by Upjohn Company, Kalamazoo, Mich.; (000011583).

Latven, A. R. (1955) Report on Acti-Dione: Chronic Toxicity Studies in Monkeys. (Unpublished study including letter dated April 2, 1956, from N. D. Conner to E. S. Feenstra, received November 5, 1956 under unknown admin. no.; prepared by Pharmacology Research, Inc., submitted by Upjohn Company, Kalamazoo, Mich.; CDL:109624-B.) (00011233)

## I. Subchronic 21-Day Dermal Toxicity (163.82-2)

The minimum data requirement for subchronic 21-day dermal toxicity is one test for the technical chemical, preferably using the albino rabbit.

A subchronic 21-day dermal toxicity test is required if pesticidal use is likely to result in repeated human skin contact.

No data are available on 21-day subchronic dermal toxicity. A 21-day subchronic dermal toxicity test may be required pending analysis of the applicator exposure and acute dermal data.

## J. Subchronic 90-Day Dermal Toxicity (163.82-3)

The minimum data requirement for subchronic 90-day dermal toxicity is one test for the technical chemical, preferably using the albino rabbit.

The subchronic 90-day dermal toxicity is required if the pesticidal use will involve purposeful application to the skin or will result in human exposure comparable to that, for example, from swimming pool additives or pesticide-impregnated fabrics.

No subchronic 90-day dermal toxicity data are available. This test is not required for cycloheximide since dermal exposure of this type should not be encountered in the normal use of cycloheximide.

## K. Subchronic Inhalation Toxicity (163.82-4)

The minimum data requirement for subchronic inhalation toxicity is one test for the technical chemical, preferably using the laboratory rat.

A subchronic inhalation toxicity test may be required pending analysis of the applicator exposure and acute dermal data.

## L. Subchronic Neurotoxicity (163.82-5)

The minimum data requirement for subchronic neurotoxicity testing is one test for the technical chemical, using either the adult hen or a mamalian species.

A subchronic neurotoxicity test is required if the pesticide has shown positive results in the acute delayed neurotoxicity in a mammalian species.

No formal subchronic neurotoxicity test data are available, however a summary of various neurotoxic effects from scientific publications has been submitted by the registrant (Johnston, R. L., 1978).

A subchronic neurotoxicity test may be combined with a subchronic oral test. When the subchronic oral test is performed, particular attention should be paid to the neurological parameters.

References:

Johnston, R. L. (1978) Safety of Cycloheximide (CHI). (Unpublished study received February 8, 1978, under 1023-50; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 098323-A.) (00012905)

## M. Chronic Feeding (163.83-1)

The minimum data requirement for chronic feeding is two tests for the technical chemical, using a rodent and nonrodent species.

A chronic feeding study is required if pesticidal use requires a tolerance or exemption from a tolerance, requires an issuance of a food additive regulation or is likely to result in repeated human exposure over a significant portion of the life span.

Cycloheximide was fed for two years in the diet to five groups of ten male and ten female rats each at 0.1, 0.5, 1.0, 2.0, and 3.0 ppm. Each level was paired with a feeding control group of five males and five females. Food consumption decreased as the concentration of cycloheximide increased. The rats at 0.1, 0.5 and 1.0 ppm begain eating as much as the controls after the second month. The rats at 2 and 3 ppm ate 60% to 90% as much as the unrestricted controls. Pneumonia was prevelant in all animals (32 of 50 rats in the paired feeding controls and 57 of 100 rats in the treated groups). Testicular atrophy was reported as 1 of 10 (1/10, 3/10, 4/10, 6/10, and 10/10 at the 0.1, 0.5. 1.0, 2.0, and 3.0 dose levels, respectively, while the nervous system was not studied since the brain and pituitary were not saved for microscopic examination.

This study has been classified as supplementary, since the incidence of pneumonia was excessive in both treated and control groups and may be obscuring toxic effects due to cycloheximide, and no central nervous tissue was examined. The brain may be an important target for cycloheximide.

The requirement for a chronic feeding study has not been satisfied; however, if the applicator and dietary exposure turn out to be sufficiently low, the subchronic feeding studies may be sufficient.

Reference:

Connor, N. D.; Runnells, R. A.; Johnston, R. L. et al. (1972) Acti-Dione (Cycloheximide) Chronic Toxicity in the Rat. Rev. (Unpublished study including letter report dated Nov. 20,1956, from N. D. Connor and R. A. Runnella to E. S. Feensdtra, received on unknown date under 2E1252; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 094789-S).

## N. Oncogenicity (163-83-2)

The minimum data requirement for oncogenicity is testing in two mammalian species, preferably the rat and mouse, using the technical chemical.

An oncogenicity test is required if the active ingredient, or any of its metabolities, degradation products or impurities are structurally related to a recognized carcinogen or cause a mutagenic effect; require a tolerance or an exemption from a tolerance, require an issuance of a food additive regulation or are likely to result in repeated human exposure over a significant portion of the life span.

Cycloheximide was fed (in the diet) at 5 and 32 ppm to ICR Upjohn strain mice for 41 weeks (Goyings, L. S., Miller, C. C., and Kaczkofsky, H. W., 1972). Each dose group (negative, control, low, high and positive control, ethyl carbamate) was made up of 50 mice (25 males and 25 females). No evidence of carcinogenicity due to cycloheximide was reported. This study was classified as invalid due to the short duration of exposure.

A second study was carried out for 79 weeks. Again only two dose levels were used; however, more mice were included in the groups (40 male and 40 female ICR Upjohn strain the each group). (Goyings, L. S., Kaczkofsky, H. W.; Kasson, C. W., 1972.)

The mice were fed cycloheximide at 5 and 32 ppm in the diet. The positive control was fed ethyl carbamate at 600 ppm. There was a decrease in body weight of the mice at the 5 and 32 ppm levels and in the mice fed ethyl carbamate. Otherwise, there were no other signs of toxicity. The incidence of tumor-bearing mice was 10%, 5%, and 5% in males and 5%, 12.5% and 2.5% for females in control, low and high dose respectively. In the positive controls (ethyl carbamate) incidence of tumor presence was 95% and 80% in males and females respectively.

Spontaneous lesions were noted in all groups and characterized as inflammatory or degenerative in nature. The lesion most frequently noted was in the urinary ststem in all groups, reflecting glomenlonephritis and/or nephritis. Cycloheximide appears to be non-carcinogenic to mice when fed in the diet at 5 and 32 ppm.

This study is classified as supplementary because no data are available concerning actual food consumption, body weight data are recorded as average body weight of five mice per cage - 8 cages per group, no individual body weights are available, no chemical analysis of food test material is presented, food with test material was prepared only once per month, and no histopathology on brain, spinal cord or bone marrow was reported.

Neither of these tests satisfy the oncogenicity data requirement. Tests in two mammalian species may be required, pending residue and mutagenicity analysis.

References:

Goyings, L. S.: Kaczkofsky, H. W.; Kasson, C. W. (1972) Cycloheximide-Oral Carcinogenic Study in ICR Mice: A Seventy-Nine Week Study. (Unpublished study received on unknown date under 2F1252; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 091784-B.) (00011609)

Goyings, L. S.; Miller, C. C.; Kaczkofsky, H. W. (1972) Cycloheximide-Oral Carcinogenic Study in ICR Mice Csicl: A

Forty-One Week Study. (Unpublished study received on unknown date under 2F1252; submitted by Upjohn Company, Kalamazoo, Mich.; CDL:094790-K.) (00011615)

O. Teratogenicity (163.83-3)

The minimum data requirement for teratogenicity is testing in two mamalian species using the technical chemical.

Teratogenicity testing is required if pesticidal use requires a tolerance or an exemption from a tolerance, requires an issuance of a food additive regulation or is likely to result in a significent exposure to females.

Three teratology studies were available but all were insufficient to satisfy the data requirement for teratogenicity testing for the following reasons:

1. Cycloheximide was administered in the food, not by oral intubation, and accurate dietary intake data were not provided. Cycloheximide has been used as a rat repellant and rats do not eat it unless they are very hungry.

2. The number of animals in each dose group was only ten, which is not sufficient.

3. Individual pup weights were never submitted, only average weights.

The three studies referred to above are:

1. Cycloheximide mouse experiment (Goying, et al., 1971). Cycloheximide was administered in the diet to six groups of 10 pregnant mice each at 0, 8, 16, 32, 64 and 125 ppm from day 6 through day 15 of gestation. No effects due to teratology were reported; however, the study was classified as supplementary for the above reasons.

2. Cycloheximide rat experiment (Goying, et al., 1971). Cycloheximide was administered in the diet to three groups of 10 pregnant rats each at 0, 0.6 and 1.0 ppm from day 6 through day 16 of gestation. No effects due to teratology were reported. The study is supplementrary for the above reasons. In addition, the highest does level was not high enough since no maternal toxicity was reported, and only two dose levels (plus a control group) were used. At least three dose levels should have been included.

3. Cycloheximide rat experiment (Goying, et al., 1970). Cycloheximide was administered in the diet to three groups of 10 pregnant rats each at 0, 0.32 and 2.0 ppm from day 6

through day 16 of gestation. Possible teratogenic effects were reported at 2.0 ppm as decreased skeletal development as 34 of 62 pups had missing sterebrae. Incomplete formation of the supreoccipital bone was reported in 7 of 62 pups. Other miscellaneous skeletal effects were reported. Two pups had anasarca and one of the two had hemothorax. Maternal toxicity (decreased body weight) was reported at the high-dose level. This study also was supplementary for the previously cited reasons.

Teratology testing by gavage in rats is required for cycloheximide, especially in view of the slight teratogenicity seen on one of the supplementary studies. No studies are available that fulfill this requirement.

References:

Goyings, L. S.; Kaczkofsky, H. W.; Bogema, L. V. et al. (1970). The Teratogenic Effects of Cyclopheximide (U-4527) in Rats. (Unpublished study including letter dated July 13, 1970, from L. S. Goyings, et al. to R. L. Johnston, received on unknown data under 1023-EX-27; submitted by Upjohn Company, Kalamazoo, Mich., CDL:126555-L.) (00014437)

Goyings, L. S.; Kaczkofsky, H. W.; Bogema, L. V. (1971) Teratology Study in Mice with Cycloheximide (U-4527)--A Final Report. (Unpublished study including a preliminary report, received on unknown date under 2F1252; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 094790-E.) (00011612)

Goyings, L. S.; Kaczkofsky, H. W.; Bogema, L. V. (1971) Teratology Study in Rats with Cycloheximide (U-4527)--A Final Report. (Unpublished study received on unknown date under 2F1252; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 094790-D.) (00011612)

## P. Reproduction (163.83-4)

The minimum dat requirement for reproduction is testing in one mammalian species, preferably the laboratory rat, using the technical chemical and lasting for two generations.

Reproduction testing is required if pesticidal use requires a tolerance or an exemption from a tolerance, requires an issuance of a food additive regulation, or is likely to result in repeated human exposure over a significant portion of the life span.

A three-generation rat reproduction study submitted in two reports (Goyings, L. S. and Kaczkofsky, H. W., 1970; and Goyings, L. S. and Kaczkofsky, H. W., 1971) was reviewed.

Four groups of 20 female and 10 male rats each were administered 0, 0.5, 0.32, and 2.0 ppm cycloheximide in the diet from weaning through breeding two litters. The 20 females and 10 males for each sucessive generation were chosen at random from these litters. It was reported that no gross abnormalities were seen in any of the groups. No reproductive effects were reported.

This study has been classified as supplementary since:

1. There were numerous discrepancies in the numbers of pups reported in the tables.

2. Litters were increased or reduced to 10 after the first 24 hours following the end of gestation, precluding viability data through weaning.

3. No gross necropsy or histopathology data were submitted.

4. No abnormalities were observed in any pups born throughout the study. Some effects would have been expected even in the absence of any treatment had the examination been sufficiently thorough.

This study does not satisfy the requirement for a reproduction study and one is necessary due to tolerance requirement for cycloheximide. It is possible that the rat is not the best animal for testing. Adverse fertilization effects were reported by Kratochivlova (1975) in the mouse, however this report was in abstract form and could not be reviewed. The registrant (Johnson, 1978) has said that a revised report will be reissued, which may enable us to resolve the above discrepancies.

## References:

Goyings, L. S.; Kaczkofsky, H. W. (1970) Effects of Cycloheximide (U4527) on Reproduction in the Rat--A Two Generation Study. (Unpublished study received December 11, 1970, under 0G0880; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 091515-C). (00011606)

Goyings, L. S.; Kaczkofsky, H. W. (1971) Effects of Cycloheximide (U4527) on Reproduction in the Rat--Report of Third-Generation of a Three-Generation Study. (Unpublished study received April 21, 1970, under 0G0880; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 0911521-AK). (00011602)

Johnson, R. L. (1978) Safety of Cycloheximide (CHI). (Unpublished study received February 8, 1978, under 1023-50; submitted by Upjohn Company, Kalamazoo, Mich.; CDL:098323-A). (00012905)

Kratochvilova, J. (1975) Pre-implantation Loss in the Dominant Lethal Assay Abstract No. 241. Mutation Research 29 (2): 216-217 (05013623).

## Q. Mutagenicity (163.84-1 through -4)

The minimum data requirement for mutagenicity testing will be finalized pending formulation of Agency Guidelines.

Cycloheximide was shown to produce mutagenic effects in the micronucleus test and the sperm abnormality assay (Bruce, W. R. and Heddle, J. A., 1979). Four groups of mice (8 mice per group) were treated with four doses of cycloheximide, i.p., on 5 consecutive days. Half the mice (females) were sacrificed after four hours and 1000 reticulocytes from femur bone marrow cells were scored for micronucleus. Sperm was examined from the remaining mice after 35 days. A dose response effect was reported for the latter micronucleus and abnormal sperm morphology. It was not possible to quantitate these effects since the exact dosage was not reported. The source and purity of the cycloheximide was not stated. These tests are adequate to show potential chromosomal effects, but more tests should be performed to verify this report. A Salmonella reverse mutation bacterial pour plate assay was also performed and reported to be negative; however, it was classified as invalid since it was not described sufficiently.

Further mutagenicity tests should be performed, to include a gene mutation assay in mammalian cells, an in vivo and in vitro cytogenetics assay, a repeat Salmonella test to include strains TA 100 and TA 98, and a cytogenetics assay designed to quantify any spindle effects that may be produced by cycloheximide.

### References:

Bruce, W. R.; Heddle, J. A. (1979) The Mutagenic Activity of 61 Agents as Determined by the Micronucleus, Samonella, and Sperm Abnormalities Assays. Canadian Journal of Genetics and Cytology 21 (3): 319-333 (05017627).

## R. Metabolism (163.85-1)

The minimum data requirement for metabolism is a single dose using the analytically pure grade of the active ingredient in the radioactively labeled form.

Two metabolism studies on rats are available, a single oral dose experiment (Petzold, E. N.; and Chapman, D. D., 1970a) and an experiment using five consecutive daily doses (Petzold, E. N.; and Chapman, D. D., 1970b).

Three male and two female rats were administered $^{14}$C labeled cycloheximide by gavage. The dosage administered was stated to be between 0.9 to 1.6 ppm. Urine and feces were collected at 24-hour intervals and the radioactive label was counted in a scintillation counter. The animals were sacrificed at 72 to 96 hours and radioactivity was measured in the blood, liver, kidney, and carcass. Nearly all the radioactivity was eliminated in the urine within 48 hours. One rat was dosed by oral gavage and its basal metabolism rate was monitored for 24-hours on a radiorespirameter. No $^{14}CO_2$ was detected.

In a second study, four female rats were administered five daily doses randomly of label $^{14}$C cycloheximide by oral gavage at 0.50 mg/kg body weight. Two of the rats had previously been receiving 2 ppm cycloheximide in the diet as part of a reproduction study. Urine feces were collected at 24-hour intervals throughout treatment and in the case of 2 rats, for 4 days post-treatment. The other two rats were sacrificed 4 hours after the fifth dosage. $^{14}$C residues were counted in the blood, brain, gastrointestinal tract, kidney, liver, muscle, abdominal fat, and the remaining carcass. Within 6 hours after the last dose, 45 to 70% of the $^{14}$C label was excreted in the urine and 75 to 80% (of the total activity of about 40%) was excreted by 4 days after treatment. Very little was seen in the organs, the highest being 269 ppb in the liver 6 hours post-treatment. All organ label levels at 923 hours post-treatment were less than 12 ppb.

These two studies suffer from the same problems. No analysis was performed of the labeled compound, so that is not known where it is labeled. No metabolities were identified, so it is not known in what form the label is excreted. Only one dose level was administered and in the single dose experiment no actual dosages were reported.

These studies were classified as supplementary and do not satisfy the requirement for a metabolism study; however, we defer the need for additional metabolism data until the dietary exposure data have been evaluated.

## References:

Petzold, E. N.; Chapman, D. D. (1970a) Excretion of $^{14}$C Labeled Cycloheximide from the Rat after Administration of a Single Oral Dose: Report No. 120-9760-14. (Unpublished study received July 28, 1970 under 1023-EX27; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 210033-M.) (00011202)

Petzold, E. N.; Chapman, D. D. (1970b) Excretion Rates and $^{14}C$ Labeled Residues on Rats Provided with Five Consecutive Daily Doses of Randomly Labeled Cycloheximide Report No. 120-976017. (Unpublished study received July 28, 1970 under 1023-EX-27; submitted by Upjohn Company, Kalamazoo, Mich.; CDL: 210033-N.) (00011203)

## S. Immunological Toxicity:

There are no minimum requirements for immunological toxicity testing.

Cooney and Bradley (1962) injected (i.p.) cycloheximide into adult BACB mice which had been immunized against actinophage M5P8 by a single injection of phage (for the early response) or by five weekly injections (for hyperimmunization). The serum was extracted, phage added, and tested for the neutralizing activity against *Streptomyces venezuela* S13, the phage host.

Cycloheximide was shown to be an effective inhibitor of both the early and late immune response. It was most effective when treatment was started several days prior to antigenic stimulation. Antibody formation resumed 24 to 48 hours after discontinuing treatment with cycloheximide.

This study is scientifically valid and is adequate to show existence of immunological suppression by cycloheximide; however, no dose response data were reported.

In view of the potential seriousness of this problem, a subset of the animals in the subchronic oral toxicity study should be examined for immunological suppression.

### Reference:

Cooney, W. J., and Bradley, S. G. (1962) Effect of Cycloheximide on the Immmune Response. Antimicrobial Agents and Chemotherapy - 1961 V1: 1-9. (000038001)

## T. Amnesia

Many investigators are using cycloheximide as a tool to produce memory loss in laboratory animals (see Johnston, 1978, for a review). All these, however, used parental routes of administration of cycloheximide so that the effects can not be quantitated for use in estimating risk to humans under actual exposure conditions. It appears reasonably certain that these effects would be manifested at higher exposure levels than other effects in the subchronic oral studies. No additional testing is required at this time.

**Reference:**

Johnston, R. L. (1978) Safety of Cycloheximide (CHI). (Unpublished study received Feburary 8, 1978, under 1023-50; submitted by Upjohn Company, Kalamazoo, Mich.; CDL:098323-A). 00012905)

## GENERIC DATA GAPS

## CYCLOHEXIMIDE TECHNICAL

| | TOPIC | REQUIREMENT | GUIDELINE SECTION |
|---|---|---|---|
| 1. | Acute Oral | One test using laboratory rat. | 163.81-1 |
| 2. | Acute dermal | One test using the albino rabbit. | 163.81-2 |
| 3. | Acute inhalation | One test using the laboratory rat including an extended observation period. | 163.81-3 |
| 4. | Primary eye irritation | One test using the albino rabbit. | 163.81-4 |
| 5. | Primary dermal irritation | One test using the albino rabbit. | 163.81-5 |
| 6. | Dermal sensitization | One test using the guinea pig. | 163-81-6 |
| 7. | Subchronic oral | Two tests using the rat and dog. | 163.82-1 |
| 8. | Subchronic 21-day dermal | Reserved: Depending on analysis of acute dermal and exposure data. | 163.82-2 |
| 9. | Subchronic inhalation | Reserved: Depending on analysis of acute inhalation and exposure data. | 163.82-4 |
| 10. | Subchronic neurotoxicity | May be combined with a subchronic oral test. | 163.82-5 |
| 11. | Chronic feeding | Reserved: Pending analysis of exposure data. | 163.83-2 |
| 12. | Oncogencity | Reserved: Not required pending residue and mutagenicity analysis. | 163.83-2 |

cont.

| | TOPIC | REQUIREMENT | GUIDELINE SECTION |
|---|---|---|---|
| 13. | Teratogenicity | One test by gavage in rats. | 163.82-3 |
| 14. | Reproduction | Revision of the three generation rat reproduction report. | 163.83-4 |
| 15. | Mutagenicity | *Five tests and a risk assessment. | 163.84-1 thru 4 |
| 16. | Metabolism | Deferred: Pending analysis of exposure data | 163.85-1 |
| 17. | Immunotoxicity | Analysis of a subset of animals in the subchronic oral toxicity study. | - |

* A gene mutation assay in mammalian cells, an _in vivo_ and an _in vitro_ cytogenetics assay, a _Salmonella_ test to include strains TA 100 and TA 98, and a cytogenetics assay designed to quantify any spindle effects that may be produced by cycloheximide.

## C. Hazard Assessment

The toxicity data currently available are inadequate to assess the potential hazard to man from the registered use patterns of use of cycloheximide products.

## D. Data Gaps

The application rate of cycloheximide is very low (40 gms/acre or less). It is possible that applicator exposure (turf and citrus use) and public dietary exposure to food resulting from the treatment of oranges may be insignificant. However, the magnitude of this exposure has not been defined. The major toxicology data requirements are an estimate of worker exposure and public dietary exposure.

Until the Agency can determine the magnitude of the exposure, the following are the minimum toxicology data requirements. These data requirements are subject to change pending the results of the Agency's review of exposure levels.

For turf use, the Agency requires an estimate of worker exposure, acute toxicity studies (for label purposes), dermal sensitization study, mutagenicity studies, a teratology study, and a 21-day rabbit dermal study designed to include both neurotoxic and immunologic parameters. The 21-day dermal study is not required if subchronic oral studies are performed.

For the citrus use, the Agency requires an estimate of dietary and an estimate of worker exposure. In addition, the following are also required: acute toxicity studies (for label purposes), a dermal sensitization study, mutagenicity studies, two subchronic oral studies, and a teratology study. A subchronic neurotoxicity test may be combined with a subchronic oral. A subset of animals in a subchronic oral test should also be analyzed for immunological suppression.

# CHAPTER VII: RESIDUE CHEMISTRY

## A. Introduction

Cycloheximide is marketed in the USA as a fungicide and a plant growth regulator. It is registered for use as a fungicide on non-bearing cherry trees (trees which will not bear fruit within 12 months), turfgrass and ornamental grasses such as golf fairways, and certain ornamental flowers, including roses. As a plant growth regulator, cycloheximide is registered for use as an abscission agent on citrus intended for processing, including oranges, grapefruit, murcotts, tangelos, tangarines, and Temple oranges in Florida only, acting to decrease the strength of attachment between the citrus fruit and twig to facilitate mechanical harvesting. Since there are no registered food uses of cycloheximide as a fungicide, this chapter addresses only the food crop use of cycloheximide as an abscission agent on citrus.

There is only one cycloheximide product registered for use on citrus. Trade named TUCO Acti-Aid®, it is marketed in a two-container unit package. Component A contains 4.22% cycloheximide and 95.78% inert ingredients. Component B contains a surfactant to be mixed with component A and water at the time of application.

Use directions specify lower concentrations of component A (cycloheximide) or higher concentrations of component B (surfactant) depending on the conditions and the type of citrus to be treated. The amount of water is also varied depending on whether the Acti-Aid® is applied by aerial or ground sprayers. Typically, a single application of 0.3 - 1.4 oz. a.i. per acre is made 4 to 7 days prior to harvest.

## B. Metabolism in Plants

Whenever a pesticide is proposed for use on agricultural crops, the Agency requires data on the fate of the pesticide in various plants. Absorption and movement characteristics of cycloheximide in plants have been studied and the following effects have been observed.

A number of early studies conducted on forest and ornamental trees demonstrated that cycloheximide is absorbed through roots, leaves and bark, is translocated both upward and downward, and persists in detectable quantities in the bark of treated trees (MRID #'s 05013629, 05015971, 05017721, 05013510, and 05013502).

Studies involving the movement of cycloheximide in food crops have also been conducted. One study on wheat seedlings demonstrated that cycloheximide was absorbed and translocated from either root or leaf application and could persist up to 5 weeks when concentrations up to 500 ppm were applied (MRID #05013890). In another study, the roots of tomato plants were exposed to cycloheximide acetate-2-$^{14}C$. Uptake into the roots, stems and leaves of the tomato plants was rapid (MRID #05026060). When rice leaves were treated with cycloheximide, translocation both upwards and downwards from the point of application was reported (MRID #0515196). Tests on corn, cowpea or cucumber, however, demonstrated no detectable movement of cycloheximide, while the same researchers found that beans (Phaseolus Vulgaris) and Montmorency sour cherries showed extensive cycloheximide movement, from roots or leaves to the beans and from leaves to cherries. The absorption was enhanced under high humidity or moisture conditions and movement appears to occur in the transpirational system. Residues found on the cherry leaves appeared to be bound within leaf tissues and persisted from 1-4 days after application (MRID #0516061, #05017469). Cycloheximide has also been shown to translocate in plum and prune trees, demonstrating phytotoxic effects in non-treated limbs (MRID #05013617), and showing loss of fungicidal activity (MRID #05013928).

In support of the registered use on citrus, there are studies which investigated the metabolism of cycloheximide in Valencia oranges. In the study conducted by Fisher (MRID #05013086), $^{14}C$-cycloheximide was painted onto the surface of oranges which were picked at intervals consistent with the application directions on the label, then rinsed, and separated into inner and outer peel, juice and rag, (remaining material) then analyzed. Most (58-83%) of the $^{14}C$ activity was rinsed off, 8-30% remained in the peel, 0.7 to 1.4% was found in the juice and 0.2 to 8.0% in the rag.

A similar study with $^{14}C$-cycloheximide was conducted by Petzold and Chapman (MRID #00012879, 00012873 and 00011227) under temperatures and humidity which approximated actual treatment to harvest conditions. When the parts of the oranges were separated and analyzed, only 0.2 ppm $^{14}C$ was detected in whole oranges. This study demonstrated that the residues of cycloheximide and its degradation products concentrate primarily in or on the peel with less than 10% penetration of $^{14}C$ labeled residues into the edible portion of the fruit.

From these studies, the metabolism of cycloheximide is adequately understood. No additional metabolism data are required.

### C. Animal Metabolism

When use of a pesticide on agricultural crops results in residues in products which may be used for animal feed, the Agency requires information on the metabolism of the pesticide in food animals.

Since dried orange pulp can be used to supplement the diet of cattle, the metabolism of $^{14}C$-cycloheximide in lactating cows has been investigated. Based upon exaggerated dosages using $^{14}C$-cycloheximide, almost all $^{14}C$ was exvreted in the urine and the feces, and there was little or no accumulation in various tissues. Residues of $^{14}C$ in milk, normalized to the expected feeding level, would be equivalent to <0.05 ppb, which would not be detectable. Approximately 13% of this was identified as cycloheximide-like substances (MRID #00011607). Although the metabolic fate of cycloheximide in animals is not fully elucidated, the available animal metabolism information is considered adequate for the need of the Agency.

### D. Analytical Methods

The general method for analysis of cycloheximide submitted by the registrant is referred to as method No. 120-9760-3. This method involves a cylinder plate bioassay designed to determine the concentration of cycloheximide by measuring the inhibition of growth demonstrated by *Saccharomyces cerevisiae*. The method was validated on whole oranges fortified with cycloheximide at 0.02 -0.25 ppm, on the processing product (molasses), but not on dried citrus pulp and meal (MRID #00011225 and MRID #00012877). The estimated sensitivity of the method for whole oranges is 0.02 ppm and for molasses is 0.08 ppm.

The specificity of the method for the determination of cycloheximide residues on oranges was tested and found to be adequate (MRID #00012873).

The method was also subjected to method try-out in EPA laboratories. It was concluded that the method is satisfactory and is suitable for enforcement of the tolerance for residues of cycloheximide on whole oranges. The method is also considered satisfactory for field trial residue data purposes.

## E. Storage Stability

The stability of residues of cycloheximide in whole or macerated oranges was investigated as an analytical control for the test method and was reported by Petzold and Chapman, 1969 (MRID #00011226, #00012831). They reported no significant decrease in cycloheximide concentration in whole or macerated oranges kept one week at room temperature or for five months in the freezer.

## F. Residues Studies of Cycloheximide

### 1. Oranges

Three studies conducted in Del Ray, Florida (2 on Valencia oranges, 1 on Hamlin oranges) on 4 trees at one location showed cycloheximide residues of <0.05 ppm following a spray application of 25 ppm cycloheximide. When a cycloheximide spray containing 50 ppm was used, residues ranged from 0.060-0.082 ppm at 1 day after application to the tree, 0.023-0.081 ppm at 4 days (after application earliest recommended harvest interval), and were not detectable after 7 days (MRID #00011228, #00011229, #00011230).

Eight additional residue studies were later provided for wider geographic and varietal differences. (MRID #00012826, #00011201, #00012825, #00011200, #00011199, #00011198, and #00011197). Five varieties of oranges at 7 locations received cycloheximide spray applications at a concentration of 20 ppm. Residues on the oranges ranged from 0.020-0.048 ppm at day zero, and by days 3-7 residues were <0.0143-0.040 ppm.

These data, all collected by the analytical method No. 120-9760-3 as previously described, are consistent with the established tolerance of 0.1 ppm for cycloheximide in or on the raw agricultural commodity citrus fruit.

Further data submissions provided additional residue data for cycloheximide on oranges. A total of 20 residue decline studies on 4 varieties of oranges in various Florida locations were submitted. In five of the studies oranges were treated with a full coverage high volume dilute spray of Acti-Aid containing 20 ppm active ingredient, applied in 500-112 gallons per acre (recommended rates) (MRID #00012847, #00012848, #00012849, #00012850, #00012846). In the 15 remaining studies, the oranges were treated with a low volume concentrate spray of Acti-Aid formulation containing 200-800 ppm active ingredient in 15-37.5 gallons of spray per acre (0.5 x 2.0 x the recommended rate) (MRID #00012851, #00012852, #00012853, #00012854, #00014438, #00012860, #00012861, #00012862, #00012863, #00012859, #00012858, #00012856, #00012855, #00014439). For those cases where treatment levels were at prescribed levels, the resulting residues were below the established tolerance of 0.1 ppm.

In eight additional studies, oranges were treated with low volume concentrate spray of the Acti-Acid formulation (0.5 x 1.5 x the recommended rate), harvested 6 days later and subjected to a post harvest aqueous wash. Residues before the wash ranged from <0.014-0.096 ppm. After the wash, approximately 50% of the residues remained. An aqueous wash is the first step in the treatment of oranges intended for the processing market. These studies indicate washing treated oranges is effective in reducing the residues on the oranges and in keeping the residues within the ranges established by the tolerance (MRID #'s 00012860, 00012861, 00012862, 00012863, 0012858, 00012857, 00012856, and 00012852).

In other studies, the effect of higher concentrations of surfactant (component B) in full coverage sprays was tested on Valencia oranges. The use of 2x concentration of component B under specified label conditions does not appear to influence the amount of cycloheximide residues (MRID #00012942).

Another report provided additional residue data for cycloheximide on oranges after aerial application in 15 gallons of water per acre. Residues of cycloheximide were between 0.017 and 0.036 ppm 0-12 days after treatment. All residues were below the 0.1 ppm tolerance (MRID #00012842).

### 2. Grapefruit

A total 12 residue studies on cycloheximide on grapefruit were reported. One trial was by aerial application of cycloheximide at 67 ppm (1 quart of Acti-Aid component A in 15 gallons of water); the remainder of trials were full coverage sprays at 10-20 ppm (0.5 to 1.0 quart Acti-Aid component A in 500 gallons water) (MRID #00014435, #00014436, #00014486, #00011533, #00011532, #00011537, #00011538, #00011534, #00011531, #00011529, #00011530, #000111535, #00011528).

These residue data for grapefruit, by Method No. 120-9760-3 dated June 27, 1969, are consistent with the 0.1 ppm toloerance.

### 3. Murcotts

For murcotts, a total of 5 studies of cycloheximide were reported, all as full coverage sprays at 10-20 ppm (0.5 to 1.0 quart Acti-Aid component A in 500 gallons water) (MRID #00011535, #000115328, #00011531, #00011538).

These residue data for murcotts, by Method No. 120-9760-3 dated June 27, 1969, are consistent with the 0.1 ppm tolerance.

### 4. Tangerines

In tangerines, a total of 8 residue trials of cycloheximide were reported, all as full coverage sprays at 10-20 ppm (0.5 to 1.0 quart Acti-Aid component A in 500 gallons water) (MRID #00011533, #00011537, #00011535, #00011528).

These residue data for tangerines, by Method No. 120-9760-3 dated June 27, 1969, are consistent with the 0.1 ppm tolerance.

### 5. Tangelos

For tangelos, a total of 6 residue studies were reported, all as full coverage sprays at 10-20 ppm (0.5 to 1.0 quart Acti-Aid component A in 500 gallons water) (MRID #00011532, #00014486, #00014436, #00011535, #00011588).

The effects of temperature and relative humidity on decline of cycloheximide residues on tangelos in environmental chambers are reported (MRID #00012878).

These residue data for tangelos, by Method No. 120-9760-3 dated June 27, 1969, are consistent with the 0.1 ppm tolerance.

### 6. Temple Oranges

For Temple oranges, a total of 5 residues studies of cycloheximide were reported, all as full coverage sprays at 10-20 ppm (0.5 to 1.0 quart Acti-Aid component A in 500 gallon water), (MRID #00011534, #00014435, #00011528, #00011535).

These residue data for Temple oranges, by Method No. 120-9760-3 dated June 27, 1969, are consistent with the 0.1 ppm tolerance.

### 7. Citrus By-Products

A study investigating the effects of processing on possible cycloheximide residues has been submitted (MRID #00011231). Oranges treated with 1x and 2x the recommended dilute spray rate (20 and 40 ppm of cycloheximide) were processed and residues of cycloheximide per se were determined in various by-products. The results of this study indicated that residues of cycloheximide will not exceed 1.0 ppm tolerance for citrus fruits and that no detectable residues of cycloheximide per se will be present in the processed by-products, dried pulp and meal, molasses and juice.

### 8. Meat and Milk

Based upon the results of the meat and milk study discussed above in Animal Metabolism and on the fact that no detectable residues of cycloheximide per se are expected in by-product feed items, it is concluded that the proposed use would be classified in category 3 of Section 180.6(a) as far as the transfer of residues of cycloheximate per se to meat and milk is concerned, provided a label restriction against the grazing of cover crops in treated orchards is retained.

## G. Summary of Data Gaps

There are currently no generic residue chemistry data gaps for the use of cycloheximide on citrus fruits.

# CHAPTER VIII: ECOLOGICAL EFFECTS

## A. Introduction

The use of cycloheximide on turf, citrus and ornamentals means that nontarget fish, wildlife and plants may be exposed to the pesticide. While much of the data available on cycloheximide can be used to help characterize its effects on nontarget species, insufficient information is available to thoroughly assess the hazard to these organisms from exposure to cycloheximide.

## B. Ecological Effects Profile

### 1. Nontarget Wildlife

### a. Technical Cycloheximide

### 1) Effects on Birds

An acute oral test using technical cycloheximide indicates that it is very highly toxic to bobwhite quail with an $LD_{50}$ of 2.59 mg/kg (MRID #00012827). This study partially satisfies the guideline requirements for effects on birds. Two subacute dietary $LC_{50}$ studies using technical cycloheximide indicate that it is at least very highly toxic to upland birds (bobwhite quail with a $LC_{50}$ of 23.4 ppm) and at least moderately toxic to waterfowl (domestic ducks with a $LC_{50}$ of 1000 ppm) (MRID #00011246 and #00011631). Due to food rejection neither study will fulfill the guideline requirement for two $LC_{50}$ studies.

### 2) Effects on Freshwater Fish

Three 96-hour freshwater fish studies with technical cycloheximide indicate that it is moderately toxic to warmwater fish (bluegill sunfish with an $LC_{50}$ of 1.3 ppm and brown bullhead with an $LC_{50}$ of 1.7 ppm) and also that it is moderately toxic to coldwater fish (rainbow trout with an $LC_{50}$ of 1.2 ppm) (MRID #00012895, #00012893, and #00012894). The bluegill and rainbow trout studies satisfy the guideline requirement for tests on two freshwater fish species.

### 3) Effects on Estuarine and Marine Organisms

The one study available on the effects of technical cycloheximide on estuarine and marine organisms indicates that it is at least moderately toxic to estuarine shrimp (the 48-hour $LC_{50}$ is 23.7 ppm) and oyster larvae with a 48-hour $LC_{50}$ of 4.8 ppm) (MRID #00011620). Neither of these studies will fulfill the guideline requirement for estuarine and marine organisms. Testing on an estuarine fish species is also required.

### 4) Effects on Nontarget Insects

Results of one study demonstrated that technical cycloheximide is relatively non-toxic to honeybees (no mortality at 241.72 micrograms/bee) when they were exposed to a direct application of a dust formulation (MRID #00036935). At this time there are no guideline requirements for tests of effects to nontarget insects, so this information is considered to be supplemental.

b. Formulated Cycloheximide (Information on the formulated product is not required, so this information is considered to be supplemental.)

Avian subacute $LC_{50}$ studies using 1% cycloheximide with a surfactant (compound B) indicated that the mixture was very highly toxic to upland birds (bobwhite quail with an $LC_50$ of 4.9 ppm) and at least moderately toxic to waterfowl (domestic duck with an $LC_{50}$ of 280 ppm) (MRID #00011246 and #00011631). Food rejection prevents either study from fulfilling any potential guideline requirement for this formulation. Three simulated avian field studies on bobwhite quail were conducted at application rates below recommended use rates and/or excess control birds died, thereby rendering these studies inadequae to fulfill any guideline requirement (MRID #00011549, #00011619, and #00012890).

Freshwater fish studies using 1% cycloheximide with a surfactant (component B) indicated that the mixture is highly toxic to warmwater fish (bluegills with an $LC_{50}$ of 0.22 ppm, and brown bullheads with an $LC_{50}$ of 0.12 ppm) and very highly toxic to coldwater fish (rainbow trout with an $LC_{50}$ of 0.075 ppm) (MRID #00012832, #00011243 and #00012833). Only the rainbow trout study would fulfill a guideline requirement for this formulation.

A test on the effects of 1.7% cycloheximide with the same surfactant (component B) indicates that mixture is at least moderately toxic to estuarine shrimp (grass shrimp with a static 48-hour $LC_{50}$ of 6.1 ppm and a flow-through 96-hour $LC_{50}$ of 7.1 ppm) and is highly toxic to oyster larvae (with an $EC_{50}$ of 0.19 ppm) (MRID #00011620). None of the studies would support any potential guideline requirement.

## 2. Nontarget Plants

The guidelines for the effects to nontarget plants specify that testing should be conducted with technical material. However, all of the studies currently available to the Agency were conducted with the Actidione formulation of cycloheximide. In addition, these studies are also typically deficient in the number and variety of plants tested. In some cases the data may provide supplemental information, but in no case do they satisfy the guideline requirements.

The following presents the supplemental information provided to the Agency through the studies with Actidione.

### a. Phytotoxicity

Studies using formulated cycloheximide indicate the no-effect-level varies considerably from greater than 3300 ppm for wahoo and butternut squash to less than 1 ppm for roses and rice (MRID #0011564 and #05016168). The phytotoxic reaction to cycloheximide as Actidione is generally manifested as a general chlorosis of the leaves. Marginal necrosis is produced at greater concentrations (MRID #05016172).

From the available vegetative vigor studies, it can be determined that the derivatives of cycloheximide (semicarbazone, methyl hydrazone, acetate, acetoacetate, thiocarbazone, oxime) are substantially less phytotoxic than cycloheximide itself (MRID #05016172, #05018300 and #05016190).

In young seedlings, applications of cycloheximide to the roots adversely affect root growth to a greater extent than similar applications of cycloheximide affect the growth of shoots (MRID #05016168). Where cycloheximide is applied to the shoots of established plants and injury occurs, the plant generally outgrows the injury (MRID #05015833, #05016168).

### b. Seed Germination

The effect of cycloheximide on seed germination varies widely with low tolerances being exhibited (0.4 in safflower and 0.05 ppm in rice) (MRID #05015967 and #05016168). No pattern to these effects can be established from the available data.

## C. Data Gaps

### 1. Nontarget Wildlife

### a. Birds

The minimum data required for establishing the toxicity of cycloheximide is one acute oral test and two avian subacute dietary tests with the technical material. The acute oral test requirement is considered satisfied as previously discussed. Available subacute dietary tests on bobwhite quail and mallard ducks are considered inadequate because the test birds refused to ingest sufficient quantities of food to permit analysis of the results. Thus the guideline requirement for a subacute dietary $LC_{50}$ for an upland game species and a wild waterfowl species have not been satisfied and they constitute data gaps. The tests should be conducted with technical grade cycloheximide on bobwhite quail and preferably on the mallard duck. A simulated field test on bobwhite quail may be substituted for the subacute dietary tests and would be preferred, if the tests are run at the highest registered application rate.

In addition to these minimum studies, the submission of an avian reproduction study is also required for cycloheximide for outdoor uses involving multiple applications.

### b. Effects on Freshwater Fish

In addition to the two minimum 96-hour studies which must be submitted and have previously been addressed, a fish embryolarvae study, preferably for rainbow trout, is also required for cycloheximide use on forests. The requirement may be made for other sites depending on environmental fate results.

### c. Effects on Freshwater Invertebrates

The minimum data required for establishing the acute toxicity of cycloheximide to freshwater invertebrates are the results from one 48-hour $EC_{50}$ or $LC_{50}$ test with technical cycloheximide on daphnids or midge larvae, or a 96-hour $LC_{50}$ test on some other freshwater aquatic invertebrate. No data were submitted for these tests and this constitutes a data gap. The 48-hour $EC_{50}$ test on *Daphnia magna* is preferred to fill this gap.

### d. Effects on Estuarine and Marine Organisms

The minimum data required for establishing the acute toxicity of cycloheximide in estuarine or marine organisms are two 96-hour $LC_{50}$ tests with technical grade cycloheximide (one test performed on shrimp species and the other test on a marine fish species, preferably sheepshead minnow) and either a 48-hour $EC_{50}$ test for oyster embryolarvae or a 96-hour shell deposition test. None of the available tests fulfill the data requirements, therefore all testing is still necessary to satisfy the requirement.

## 2. Nontarget Plants

Plant hazard assessments are no longer routinely being made.

## D. Hazard Assessment

### 1. Nontarget Wildlife

Most of the cycloheximide produced each year is used as a fungicide on turf in the northern states. A small portion of the cycloheximide produced is used as an abscission agent on Florida citrus. These uses may result in exposure to both terrestrial and aquatic organisms, including marine and estuarine species. The absence of environmental fate data on persistence and transport preclude a complete nontarget wildlife hazard assessment at this time. Extrapolation of a one-day dietary $LC_{50}$ from the technical avian acute oral and supplemental data on the subacute dietary for the cycloheximide formulation indicate that residues from citrus and turf use (4.5 to 26.4 ppm on short grasses) are hazardous to birds. Insufficient use rate data are available to estimate residues on ornamentals, citrus, non-bearing cherry trees, and other tree species, so a similar analysis cannot be conducted for these uses. Evaluation of avian hazard was further complicated by avian dietary studies which are considered inadequate because of excessive food rejection, and poor results from three simulated avian field tests which were inconclusive due to disease, control group mortality, and application rates below registered rates.

Acute fish toxicity data indicate that there is no apparent acute hazard from a single application at reported application rates (0.3 to 1.8 oz a.i./A) for turf or 0.3 - 1.4 oz a.i. per acre for citrus. Acute and chronic hazard from multiple applications to turf, trees, ornamentals, and non-bearing cherry trees cannot be assessed until persistence and transport data are available.

Evaluation of possible chronic toxicity effects on fish and birds from multiple applications must also await the results of environmental fate data and the requested reproduction studies.

Application to turf and citrus groves may affect adjacent estuarine and marine areas. Golf courses are frequently associated with streams, ponds, and lakes which could be contaminated with cycloheximide by runoff. Since citrus crops in Florida cover about 910,924 acres, expansion of the presently negligible use in citrus would greatly increase the likelihood for exposure to freshwater and estuarine areas. Central Florida is a major citrus-growing area and the area is dominated by numerous freshwater lakes of various sizes which are virtually surrounded by citrus groves. In the coastal areas, such as the

Indian River region, Dade County and the central Gulf Coast, there are 304,980 acres of citrus which are located near and may impact on estuarine and marine environments. Estuarine toxicity testing data must be submitted before any hazard assessment can be made.

## E. Summary of Major Data Gaps

The major data gaps for nontarget wildlife are two avian subacute dietary $LC_{50}$ tests on bobwhite quail and preferably mallard duck or a simulated avian field study on bobwhite quail at the highest registered application rate; an acute freshwater invertebrate test, preferably on *Daphnia*; and acute toxicity studies on estuarine and marine organisms as listed in the Table in Chapter III. Uses such as turf, ornamentals, forests, and non-bearing cherry trees, which involve multiple applications also require the submission of an avian reproduction study on an upland speices, preferably bobwhite quail, and a fish embryolarvae test, preferably on rainbow trout.

# IX. CASE BIBLIOGRAPHY

## Guide to Use of This Bibliography

1. Content of Bibliography. This bibliography contains citations of all the studies reviewed by EPA in arriving at the positions and conclusions stated elsewhere in the Standard. The bibliography is divided into two sections: (1) citations that contributed information useful to the review of the chemical and considered to be part of the data base supporting registrations under the Standard, and (2) citations examined and judged to be inappropriate for use in developing the Standard. This second part of the bibliography exists in the Agency's files and does not accompany this Standard. Interested parties may request a copy from the Agency. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions, and the published technical literature.

2. Units of Entry. The unit of entry in this bibliography is called a "study." In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review, and can be described with a conventional bibliographic citation. The Agency has attempted also to unite basic documents and commentaries upon them, treating them as a single study.

3. Identification of Entries. The entries in this bibliography are sorted by author, date of the document, and title. Each entry bears, to the left of the citation proper, an eight-digit numeric identifier. This number is unique to the citations, and should be used at any time specific reference is required. This number is called the "Master Record Identifier," or "MRID." It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted data; see paragraph 4(d)(4) below for a further explanation. In a few cases, entries added to the bibliography late in the review may be preceded by an eight-character temporary identifier. This is also to be used whenever specific reference is needed.

4. Form of the Entry. In addition to the Master Record Identifier (MRID), each entry consist of a bibliography citation containing standard elements followed, in the case of materials submitted to EPA, by a description of the earliest known submission. The bibliographic conventions used reflect the standards of the American National Standards Institute (ANSI), expanded to provide for certain special needs. Some explanatory notes of specific elements follow:

   a. Author. Whenever the Agency could confidently identify one, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as author. As a last resort, the Agency has shown the first known submitter as author.

b. Document Date. When the date appears as four digits with no question marks, the Agency took it directly from the document. When a four-digit date is followed by a question mark, the bibliographer deduced the date from evidence in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c. Title. This is the third element in the citation. In some cases it has been necessary for Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. Trailing Parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission Date. Immediately following the word 'received' appears the date of the earliest known submission.

(2) Administrative Number. The next element, immediately following the word 'under,' is the registration number, experimental permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is th submitter, following the phrase 'submitted by.' When authorship is defaulted to the submitter, this element is omitted.

(4) Volume Identification. The final element in the training parenthesis identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol 'CDL', standing for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume. For example, within accession number 123456, the first study would be 123456-A; the second, 123456-B; the 26th, 123456-Z; and the 27th, 123456-AA.

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